THE EFFECT OF GROWTH REGULATORS AND ENVIRONMENTAL FACTORS ON BUD AND SEED DORMANCY OF *PRUNUS PERSICA*

By

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INTRODUCTION

It has been known for many years that deciduous fruit trees at certain stages of growth and development pass through a period of rest, a period of time during which a plant or plant part will not grow regardless of how favorable environmental conditions may be. The entrance into and emergance from this resting condition is gradual. This applies to the dormancy of embryos as well as buds. In the case of many woody plants in a temperate climate, the resumption of growth depends upon preconditioning the tissue with low temperatures. In this report, the type of bud or seed dormancy which is terminated by a period of low temperatures will be referred to as "rest".

The fundamental and practical importance of understanding the nature of this rest period has prompted many investigations but at the present time very little is known of the physiological mechanism involved in this phenomenon. Recently, a very useful tool for studying plant dormancy has become available--the gibberellins. Of more than 126 compounds known to influence dormancy to varying degrees (29), gibberellic acid has been found to be the most effective agent for inducing growth. In the case of certain tissues, it seems to overcome entirely the chilling requirement (67). This has led to the suggestion that gibberellins may play a natural role in controlling dormancy. To add further support to this idea, gibberellin-like substances have been found in higher plants. This makes it reasonable to consider that a gibberellin-like factor may be involved in the dormancy of plants. In case gibberellins are found not to be involved in the natural control of the rest period, an understanding of the relation of the substances to dormancy could help to shed some light on the natural mechanism controlling this phenomenon.

In the course of this study, three approaches to the problem were taken. First, experiments were designed to determine if there was an interaction between gibberellins, auxins and auxin-antagonists on peach bud and seed dormancy. Second, an attempt was made to isolate gibberellin-like substances from organs in different stages of growth and development to determine if there existed a correlation between the amount of substances in the tissue and the growth capacity of the organ. Third, experiments were conducted to determine the effect of high temperatures and anaerobiosis on peach bud and seed dormancy.

LITERATURE REVIEW

The Relation of Gibberellins to the Dormancy of Seed and Buds

Dormancy is of quite common occurrence in the plant kingdom. In many plant organs, periods of growth alternate with periods in which little growth takes place. The seeds of many plants germinate only after they have been exposed to certain light or temperature conditions before being placed in an environment normally favorable for growth. This state of inhibited growth of seeds or other plant organs as a result of internal causes is known as "dormancy". In nature, this condition is environmentally controlled and is obviously of adaptive value.

In this brief review on dormancy and gibberellins no attempt has been made to cover completely all the rapidly growing literature. Papers have been cited which seem especially pertinent. Several excellent reviews on bud and seed dormancy (29, 77, 85, 22) and on gibberellins (83, 14) have been published.

Bud dormancy

We are all familiar with the dormancy of deciduous trees in temperate regions. Many types of plant buds will not resume growth in the spring unless they have had a sufficient amount of chilling during the winter. The duration of low temperature required varies from one species to another, ranging from a few days to several months at a temperature of 0° to 10° C.

A great deal of research has been done on the nature of dormancy and the means of breaking it. Some chemicals, notably, auxins, gibberellins, glutathione, substituted phenols, etc., have been found to be effective in breaking bud dormancy to varying degrees. Other treatments such as X-ray, radium emanation, and warm water baths have also been reported to break dormancy. In case of both chemical and physical treatments, gibberellins have been found to have the most pronounced effect on overcoming bud dormancy.

The influence of gibberellins on dormancy, coupled with observations on the relation between growth regulators and breaking of the rest period, indicates that this state of arrested growth may be hormonally controlled.

There appear to be two main thoughts which have emerged regarding the hormonal concept of the control of dormancy. The first is on the role of auxin. Jensen (52) suggested that dormancy may be due to a lack of auxin in plant tissue. In this regard, Zimmerman (95) reported auxin only in sprouted buds, and Avery, Burkholder and Creighton (3) could find no auxin in dormant shoots of Aesculus and Malus.

On the other hand, Guthrie (39) suggested on the basis of his work that bud dormancy may be due to high concentrations of auxin. He observed that the application of naphthaleneacetic acid (NAA; see Table 1 for abbreviations) to potato tubers resulted in prolonged

TABLE 1

A LIST OF ABBREVIATIONS OF CHEMICALS USED IN THIS REPORT

Chemical	Abbreviation
Gibberellic Acid	GA
Indole-3-acetic Acid	IAA
Maleic Hydrazide	MH
Naphthaleneacetic Acid	NAA

dormancy. Furthermore, Eggert (30) reported that dormancy of apple buds terminated only when their auxin content dropped below 0.25 micrograms.

The second hypothesis put forth is on the role of plant growth inhibitors. Hemberg has been the strongest advocate of the idea that growth inhibitors are responsible for imposing bud dormancy. He demonstrated that growth inhibitors are present in dormant buds of Fraxinus (45, 48) and in the peel of resting potatoes (44, 46, 47, 49), and that growth inhibitors disappeared with the termination of the rest period. Blommaert (8) has also shown the presence of acid-inhibiting substances in dormant peach buds. These inhibitors also lessened with the termination of dormancy.

In a continuation of this work, Blommaert (9) extracted peach buds which were subjected to different cold and warm dormant periods. He found that the content of growth inhibitors disappeared more rapidly in trees which were provided with a relatively long cold period. Moreover, Biggs (7) made extracts of non-dormant peach buds and reported the presence of several growth promoters as well as several growth inhibitors. Hendershott and Walker (50) obtained growth promoters and growth inhibitors from the dormant flower buds of peaches. Inhibitors were present in a much larger quantity in dormant than in non-dormant buds.

The role of gibberellins on dormancy has also been investigated. It has been reported that gibberellins may be involved directly or indirectly in dormancy. Several investigators have found these substances in rapidly growing organs (90, 76, 25, 82). Others have found that gibberellins will break the rest period of many plant organs (22). Since gibberellins have been used extensively in this research program, a rather comprehensive review of the relation of gibberellins to both bud and seed dormancy has been presented.

Seed dormancy

The freshly harvested seed of many plants will not germinate even when put under environmental conditions favorable for growth. Such seeds are known as dormant. In some cases, seed dormancy is caused by the impermeability of the seed coats to water or oxygen, or to the presence of rudimentary embryos. In other cases, physiological conditions internal to the seed cause dormancy which is not of a transport nature, and is overcome by light or temperature. Apple, peach, pear and plum seeds have this latter type of dormancy which is broken by low temperature conditions.

In addition to the above, a number of plants exhibit a special type of dormancy. The seeds are capable of sprouting but the resulting seedlings will have an abnormal type of growth. This type of dormancy can be overcome by certain temperature and light treatments.

It has been suggested on the basis of many published reports that hormones are responsible for seed dormancy, and that the mechanisms may be similar to that of buds. In the case of peach and other cold-requiring seeds it seems that the tissue at low temperature can effect a balance of growth regulators which will stimulate growth and development when the tissue is returned to an environment favorable for growth.

Many growth regulators of an inhibitive nature have been reported to be present in seeds. Evenari (31) has listed 15 natural germination inhibitors present in seeds. Some of the most effective are coumarin, dehydroacetic acid, ferulic acid, parasorbic acid and the phthalids. In the same review, there were listed over 100 species of plants from which germination inhibitors have been isolated. In more than 60 of the species, the inhibitors were found in seeds or fruits, and in 22 species seeds were specifically mentioned as the source. As an example of the investigations included in this compilation, Randolph and Cox (73) found growth inhibitors in the endosperm of Iris; Akamine (1) observed the presence of inhibitors in the floral parts of Pennisetum ciliare. In the latter case the inhibitors were found in the bristles, glumes, lemmas, paleas, and other structures external to the seed but adhering to it.

Recently, Blumenthal-Goldschmidt (10), working with seeds of lettuce, reported that the dry seeds contained inhibitors. Also Yoshidu (94) demonstrated that growth promotors increased and growth inhibitors decreased with increases in the amount of chilling of Abies sachalinensis fr. Sachmn. seeds. Inhibitors and promotors have also been found in dormant as well as non-dormant peach seeds by Flemion and de Silva (34).

It remains an enigma to the concept of inhibitors controlling seed dormancy that 2,4-dichlorophenoxyacetic acid and maleic hydrazide (MH) are relatively inactive as inhibitors of seed germination when exogenously applied. Working with seeds of wheat, beans and other plants, Everson and Dunham (33) reported that a measurable effect of 2,4-dichlorophenoxyacetic acid was obtained only at concentrations greater than 10^{-3} M. There is a possibility that the externally applied growth regulators may not be reaching the site of action involved with the onset of germination.

Since it has been suggested that physiological responses of plants to auxin may depend in part on sulfhydryl compounds, the assumption that hormones may be involved in dormancy has been further substantiated by the fact that certain compounds having a -SH group such as glutathione, cysteine, and 1-2-dithiopropanol are promotors of seed germination. Elliot and Leopold (32) observed that an inhibitor of germination and elongation found in seed coats of Victory oats was reduced in its action by 1-2-dithiopropanol and glutathione. These two compounds were also found similarly effective on reversing the inhibition of Avena-section lengthening by coumarin and protoanemonin (84).

The Physiological Action of Gibberellins

Gibberellins were first isolated from the fungus Gibberella fujikoroi Saw. Since their discovery, many reports have been made of the influence of these substances on plant growth and development, e.g., cell elongation and division, flowering, bud and seed dormancy, etc.

Bud dormancy

The breaking of bud dormancy is an interesting effect of gibberellic acid (GA), since it amounts to the induction of new growth. Some plants, such as peach, require winter chilling before they will resume growth in the spring; many ornamentals grow slowly when days are short or the temperatures are too low; lawn grasses do not reach optimum growth until summer temperatures are attained. In such instances, the application of gibberellins appears to overcome most of this natural inhibition of growth.

Brian, et al. (18) observed that gibberellins would break the dormancy of buds on potato tubers. Lang (56) reported that the plants of a non-cold-treated species of a biennial strain of henbane, Hyocyamus niger, were forced to bolt by the application of gibberellins to the center of the rosette. Barton and Chandler (6) have reported the breaking of epicotyl dormancy of tree peony seedlings by the application of GA to the hypocotyl of young seedlings. Also, GA was effective in breaking the dormancy of peony seedlings not receiving low temperature treatment and in promoting elongation of seedlings which had received low temperature treatments.

Furthermore, Donoho and Walker (28) found that gibberellins greatly influenced bud dormancy of peach trees. Their test material was two-year-old Elberta peach trees which had received 164 hours of temperature below 45° F. After this partial dose of chilling, the trees were treated with various concentrations of GA and placed in the greenhouse. Under these conditions, they reported that four weeks after the GA treatment the plants which were sprayed with greater than 500 ppm GA had resumed growth, while the plants sprayed with lower concentrations of GA and distilled water were still dormant. With another group of Elberta peach trees (which were given 433 hours of chilling at 45° F), dosages of 100 and 200 ppm proved effective in breaking dormancy. Further work by Walker and when days are short or the temperatures are too low; lawn grasses do not reach optimum growth until summer temperatures are attained. In such instances, the application of gibberellins appears to overcome most of this natural inhibition of growth.

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Donoho (87) indicates that different storage temperatures before GA treatment had no effect on rest curtailment when 500 and 1000 ppm were applied to peach trees. However, with low concentrations, trees kept at 40° F resumed growth earlier than those kept at 65° F for a similar period of time.

Lippert, et al. (57) found a curtailment of the rest period, resulting from treatment with GA, of newly dug White Rose, Kenneb and Russet Burbank potatoes. There was a two to three weeks acceleration of sprouting. Lona and Borghi (59) stated that 40 per cent of the beech buds were forced to swell when winter twigs were placed for 15 days in 1:20,000 GA solution, compared with 20 per cent of similar buds from twigs in distilled water. Recently, Brown, et al. (19) have reported the effect of gibberellins on pear buds. They applied GA to pear trees at different times during a dormant season. Some of the treated trees were left outside while the others were moved into the greenhouse. Bud stimulation by GA treatment was reported only in trees moved into the greenhouse which had been given partial chilling before they were treated. The authors suggested that GA does not seem to be responsible for breaking rest in pear buds, but rather stimulates the growth of buds which have emerged at least primarily from rest.

Cooper and Reynado (24) applied GA in several concentrations to the foliage of the grapefruit trees. They reported that in some instances elongation of shoots was observed. Also, Ga induced flowering in Azalea plants which had not received any low temperature treatments (11, 62).

High concentrations of gibberellins may be inhibitory and produce abnormalities. Bradley and Crane (12) observed that the application of GA in relatively high physiological concentrations to the branches of Prunus species inhibited the growth and development of both vegetative and flower buds.

Since plant dwarfism and dormancy have characteristics which are strikingly similar, a brief look at the influence of gibberellins on dwarfism may prove fruitful. Brian and Hemming (15) were the first to report the unusual response of dwarf varieties of plants to gibberellins. They found that a few micrograms of GA would increase the growth rate of dwarf peas to that of a non-dwarf variety. The following year, Barton (5) showed that an application of GA, but not auxin, doubled the growth rate of dwarf Malus arnoldiana Sarg. However, the growth rate was not comparable to that of normal seedlings treated with GA.

Bukovac and Wittwer (20) also reported that a striking response to the gibberellins was obtained with bush beans which, when treated, formed twining vines with growth characteristics that resembled pole-beans. It should also be reported that the rosette condition of some plants, which is caused by long days, is reversed by GA (56). This reversal of rosetting can be thought of as releasing environmentally induced dwarfing.

It should also be stated that the application of gibberellins induced growth in non-dwarf plants also. Marth, *et al*. (61) reported the influence of GA on 49 kinds of plants, included were various genera, species, varieties and hybrids of cultivated and non-cultivated plants. They found that most of the plants responded to GA treatment in various ways but the most pronounced was stem elongation. Marked differences between the responses of one kind of plant and another were noticed. Greatest stem elongation resulted when the chemical was applied to stems that had just begun to elongate. Others have reported similar findings. Cooper (23) observed that the application of a 1 per cent solution of GA resulted in increased shoot elongation and induced growth in lateral buds on new shoots. Rappaport (74) obtained growth stimulation in tomato plants by the application of GA at concentrations of 100 and 200 micrograms, but not by concentrations of 25 or 50 micrograms per plant.

With this mounting evidence, it seems logical to assume that gibberellin can influence some basic step or steps in metabolism so as to increase the growth capacity of many plants.

Seed dormancy

Because of the manifold evidence that bud and seed dormancy are physiologically similar, a review of the effects of gibberellins on seed dormancy could shed some light on the problem.

Many seeds require a period of exposure to low temperature, to light, or to some other factor or combination of factors before they will germinate. The preconditioning requirements of seeds before they will germinate have been eliminated in certain cases by gibberellins.

Lona (58) first reported the stimulation of lettuce seed germination by gibberellins. Later, Kahn, et al. (53) reported that a mixture of gibberellin A_1 and gibberellin A_3 permits the germination in the dark of lettuce seeds possessing a natural requirement of red light for germination. This mixture also promoted the dark germination of other seeds which have been shown to have a dependency on red light for germination (55).

The acceleration of germination of cold-requiring seeds by gibberellins has been just as pronounced. Donoho and Walker (28) gave a partial chilling treatment of 35 days to peach seeds before soaking them for 24 hours in 0, 20, 100, 200, 500, and 1000 ppm of GA. After the GA treatments, the seeds were sown in flats of sand. The results indicated that concentrations of 100 and 200 ppm GA stimulated germination more than any other concentration used, and that concentrations of 500 to 1000 ppm GA may have been toxic for optimum germination. Gray (38) also reported that the application of gibberellin was found to replace the cold requirement for the germination of peach seeds. Other seeds, known to require chilling for germination, have exhibited similar responses to gibberellins.

Non-dormant seeds are also affected by gibberellins; Hayashi (41) showed long ago that these substances accelerated the germination of barley, wheat and rice seeds. Ching and Stewart (21) reported that seeds of papaya, orchids and various other varieties of native California annuals responded to GA treatment. Also, the ability of gibberellins to promote the sprouting of many weed seeds (21), wild oats (43), beans, peas and sweet corn (92) have been reported. Nevertheless, the use of gibberellins in the study of seed germination, especially the cold-requiring phase of certain seeds, could give new insights into the problem of mechanisms controlling growth.

Interaction between gibberellins, auxins, and auxin-antagonists

✓ As has been previously stated (36), the growth of a plant must depend in part on the interaction of native gibberellins, auxins, auxin-antagonists, vitamins, kinins, and other as yet unknown growth regulating substances. Limiting amounts of any one or more of these could be the factor for a single or multiple requirements for inducing growth.

Evidence exists which indicates interaction between gibberellins and auxins (68, 13, 16, 54, 91, 88) occurs in tissues. It has been suggested that the effectiveness of gibberellins as growth regulators depends on the presence of endogenous auxin (42).

Nitsch and Nitsch (68), using coleoptiles and the first internodes of oats, observed that a mixture of GA and indoleacetic acid (IAA) showed less than additive growth response when compared to either alone, but the two in combinations did have a synergistic response. Also, Brian (13) observed that the growth of pea cuttings treated with GA and IAA indicate an interaction between the two substances. The plants attained greatest height in 10^{-5} M IAA than in either alone or in any other combination tested.

Furthermore, Kato (54) reported IAA to be more effective than GA in inducing better growth of pea sections, but the small effect of gibberellins was additive to the IAA or NAA responses at all concentrations. In 1958, Radley (71) reported some interesting results on the growth of sections of etiolated wheat seedlings. She observed that sections cut 4 mm from the base of a node, having a high endogenous growth rate, responded to gibberellins with and without additional application of IAA. The sections taken 12 mm from the base of a node, having a low endogenous growth rate, gave no response to IAA or GA alone, but responded when both were applied.

More evidence of an interaction between GA and IAA has been obtained by Vlitos and Meudt (86), and Brian and Hemming (17). When a decapitated pea plant is treated with GA there is practically no extension of the sub-apical internodes, suggesting that something is produced in the apex which is necessary for the GA response.

Hayashi and Murakami (42) could not obtain synergism on growth between IAA and GA in tissues limited by substrates. When epicotyl sections of peas were placed in distilled water for 18 hours prior to treatment, the tissue did not respond to GA, but responded to IAA. However, when sucrose was added to the medium the explants responded to GA also. Moreover, there was a synergism on growth between GA and IAA only in the presence of sucrose.

An attempt to obtain synergism between gibberellins and auxins on intact plants has also been reported. Yabuta, et al. (93) applied GA in various combinations with auxin to rice seedlings, but their results were not consistent enough to draw any conclusions. ✓Later, Kato (54) observed that the growth inhibition of cucumber plant due to IAA and NAA applications was reduced by gibberellins.

A few reports have also appeared on the interaction between gibberellins and auxin-antagonists. Riddell (75) found that application of GA would reverse the inhibition of growth of tomato plants by MH. This reversal was the most pronounced when the MH application was only 500 ppm and GA was applied one week after the MH spray.

Recently, Haber and White (40) stated that they could obtain no specific interaction between GA and MH on lettuce seed germination and wheat seedling growth. The action of MH seems to be through inhibition of cell division rather than inhibition of cell elongation.

The Occurence of Gibberellins in Higher Plants

Evidence seems to clearly indicate that gibberellins or gibberellin-like factors occur in higher plants. Mitchell, *et al*. (64) obtained an ether-soluble extract from immature seeds of *Phaseolus vulgaris* var. Black Valentine which accelerated vegetative growth when applied to seedling plants of the same variety. In 1956 Radley (70) reported gibberellin-like activity from extracts made from young shoots of dwarf and tall varieties of peas. The following year West and Phinney (90) made extracts from the seed or fruit of a number of flowering plants which stimulated plant growth in a manner comparable to that obtained with gibberellins isolated from *Gibberella fujikuroi* Saw.

Using the maize dwarf-1 mutant bioassay, Ritzel (76) found gibberellin-like activity in seed or fruit of 20 species from 15 different genera. The activity was obtained from acetone: water (1:1) diffusates of the seed, fruits or the crushed endosperm. In all cases the activity increased with increasing seed size until the seeds approached maturity. Then the activity in the diffusates decreased. It is interesting to note that the increase in gibberellin-like activity occurred during the time of the maximum growth of the embryos. In this same report no activity was found in the seeds of 35 other species tested.

McComb and Carr (63) made extracts from peas with ethyl acetate which showed gibberellin-like activity. The extracts were purified by chromatographic means and then assayed using dwarf pea seedlings. Phinney and Neely (69) also obtained gibberellin-like activity from extracts isolated from beans and peas.

Radley (71) has reported the presence of gibberellin-like substances from ethanol extracts of all parts of tall and dwarf pea seedlings, in immature runner bean seeds and in mature seeds of tall and dwarf peas, wheat and french beans. She used a wheat leaf test to screen for activity in the extracts. Later, Radley and Dear (72) obtained gibberellin-like substances from the milk, endosperms and embryos of coconut. Simpson (80) also found highly significant amounts of gibberellin-like substances in extracts obtained from the nodes and tips of wheat seedlings.

Furthermore, Corcoran (25), using a dwarf mutant of maize as the bioassay, obtained evidence of gibberellin-like substances from acetone extracts of young seed or fruit of 35 species of flowering plants representing the 20 genera: Acacia, Astrogulus, Benincosa, Calendula, Casimiroa, Cassis, Cleome, Coreopsis, Cucumis, Cytisus, Gazania, Helianthus, Lupinus, Passiflora, Phaseolus, Prunus, Retana, Thermopsis, Ulex and Wisteria. Murakami (65) also obtained evidence of gibberellin-like substances in 15 species of Leguminosae.

Recently, Smith and Rappaport (82) made extracts from the peel of potato tubers which were stored at 20° C and sampled at 12-day intervals until sprouts appeared. The extracts were purified, chromatographed and were tested by Phinney's dwarf-5 maize bioassay. The results indicated that the activity of gibberellin-like substances was low in the first three samples but increased near the time of sprouting.

The occurrence of gibberellins in higher plants has been substantiated further by two isolations of dihydrogibberellic acid from beans (89, 60).

Dormancy of seeds and buds seems to be hormonally controlled. The most prevelant concepts on the mechanisms controlling dormancy are that it may be due either to a super- or sub-optimal growth hormone content or to naturally occurring growth inhibitors.

Out of the many chemical and physical treatments which influence the rest periods of plant organs, gibberellins have the most pronounced effect on overcoming dormancy, except for the natural means by which dormancy is broken, e.g., light, exposure to low temperatures, etc.

THE INFLUENCE OF GIBBERELLIC ACID, NAPHTHALENEACETIC ACID, INDOLE-3-ACETIC ACID AND MALEIC HYDRAZIDE ON BUD DORMANCY

Introduction

Dormancy is of quite common occurrence in the plant kingdom. Certain plants, e.g., plum and peach have a rest period that is broken in nature by an extended period of low temperatures. It has been suggested that plant growth regulators, particularly the phytohormones, may be responsible for dormancy. There exists a great deal of indirect evidence to support this concept (cf. literature review, pages 3 to 19), but little direct evidence. To gain further knowledge of the role of growth regulators in controlling bud dormancy of woody plants, an investigation was made of a possible interaction between low temperature and three growth regulators, namely gibberellic acid (GA), naphthaleneacetic acid (NAA), indole-3-acetic acid (IAA) and maleic hydrazide (MH), on this block to growth.

Materials and Methods

Influence of chilling and gibberellic acid on Lovell peach buds

In an attempt to establish some trends as to the influence of chilling and GA on dormant and non-dormant buds and to determine the approximate concentration range of growth regulators to be used in later experiments, preliminary experiments were conducted with seedlings of Prunus persica var. Lovell.

The seedlings used in these experiments were kept in a local greenhouse for the duration of the tests except for a period of time in the refrigerator. At the time the plants were given low temperature treatments, they were five-month-old seedlings. To maintain vigorously growing seedlings, they were pinched back two months before the cold treatment.

Seedlings were kept in a walk-in refrigerator, maintained at $7^{\circ} \pm 1^{\circ}$ C for varying lengths of time from 0 to 1200 hours. Immediately after the exposure to low temperature, the plants were treated with one of several concentrations of GA. Fifteen seedlings were used per treatment.

The growth regulator was incorporated into lanolin and applied at the rate of 0.2 ml per seedling on the sides of the stem about 5 cm below the apex. Care was taken to keep the area covered by the lanolin the same. The growth by elongation of the stem of each seedling was recorded at regular intervals until they became dormant.

Approximately three months from the termination of the above experiment, the dormant seedlings were used again to determine the effects of low temperature and GA on the termination of the rest period of buds. Fifteen seedlings were used per treatment and were carefully selected to include plants that were of comparable height and that had become dormant about the same time. The method of seedling treatment was the same as in the first experiment.

Data were taken on the number of seedlings resuming growth and the total number of shoots and suckers.

Interactions between low temperature, gibberellic acid, naphthaleneacetic acid, and maleic hydrazide on bud-break and the growth capacity of peach buds

In order to have buds homozygous in their requirement for chilling to terminate the rest period, peach plants were obtained by asexual propagation from *Prunus persica* var. Okinawa, according to the methods of Sharpe (81). After the cuttings were rooted, they were planted in four-inch plastic pots in a sandy loam and peat mixture (1:1). Approximately three months after potting, these plants were used to determine the influence of low temperature, GA, NAA and MH on the termination of dormancy and to determine if there were any interactions between low temperature, GA, NAA and MH on bud dormancy.

When the influence of low temperature, GA, NAA and MH on bud dormancy was studied, the dormant plants were exposed to a temperature of $7^{\circ} \pm 1^{\circ}$ C from 0 to 528 hours, before the chemical was applied. To accomplish this, samples of plants were periodically removed from the low temperature environment and treated with lanolin, plus or minus the addenda. The method of application was the same as in the preliminary experiments.

The number of cuttings used per treatment was ten, and the number of vegetative buds on each plant varied from one to six. Data were taken on the number of buds terminating dormancy and on the elongation of the main shoots. These data were analyzed by the "factorial chi square" method.

When interactions between low temperature, GA, NAA and MH were studied, all plants received 240 hours of a 7° ± 1° C temperature pretreatment. Following chilling, the treatments were the same as in the foregoing experiment.

The influence of gibberellic acid and indole-3-acetic acid on the termination of dormancy of peach buds

An additional experiment was conducted on asexually propagated one-year-old dormant peach plants of the Okinawa variety. A series of concentrations of GA and IAA were applied by injecting the test solutions directly into the bud scales on the apex of the bud (0.05 ml per bud). Ten plants, which had from five to ten buds on the last flush of growth, were used per treatment. Data were recorded on the number of buds terminating dormancy.

Experimental Results

Influence of low temperatures and gibberellic acid on bud dormancy of Lovell peach seedlings

For the initial experiments on the influence of temperature and GA on bud dormancy, it was decided to use peach seedlings with an actively growing apical bud. It was thought that the low temperature would first force the plants to go dormant and then additional time in a low temperature would satisfy the chilling requirement needed to break dormancy. If this were the case, the effects of GA could be determined on both the resumption and termination of bud dormancy. Contrary to this idea, low temperature did not force the seedlings to go dormant. In fact, low temperature seemed to increase the growth capacity of the seedlings when returned to an environment favorable for growth, as can be seen in Table 2. Compare the growth as influenced by the 7° C treatment in the controls after 30 days in the greenhouse, noting also the influence of GA. At ten days from treatment high concentrations of GA promoted elongation where no (0 hours) or very little (120 hours) chilling was given. With higher dosages of chilling, GA had no influence on growth. At 20 days from treatment, GA in high concentration promoted growth comparable to 360 hours of chilling, after which it had no additional effect. A similar trend continued at 30 days. In general, it would seem that both the higher concentrations of GA and pretreatment with low temperature has approximately the same effect on the growth capacity of the buds.

Since low temperatures did not force the seedlings to go dormant, a similar experiment was conducted using plants that had entered the dormant stage under natural fall weather conditions. The data are presented in Fig. 1. It is clearly evident that the chilling greatly influenced the bud dormancy of the seedlings. Three weeks of chilling had the most pronounced effect on the resumption of bud growth. Under the conditions of this test GA had only a slight effect on breaking bud dormancy.

Further data from this experiment showed that the growth capacity (maximum elongation) of the seedlings increased with increases in the length of the chilling period up to seven weeks. Periods of chilling longer than seven weeks had an adverse effect on the growth capacity of the seedlings. This can be seen in Fig. 2.

TABLE 2

EFFECT OF GIBBERELLIC ACID ON STEM ELONGATION (CM) OF NON-DORMANT SEEDLINGS

Hours of pretreatment at low temperature	Days after removal from 7° C											
	10				20				30			
	Control	GA			Control	GA			Control	GA		
		10^{-5}M	10^{-4}	10^{-3}		10^{-5}	10^{-4}	10^{-3}		10^{-5}	10^{-4}	10^{-3}
0	5.3	5.6	8.9	11.4	6.0	7.5	10.7	14.2	6.6	7.5	10.9	14.7
120	4.5	5.2	5.1	8.7	9.3	10.5	12.0	14.9	12.8	14.7	14.5	17.0
240	6.0	5.7	5.3	7.3	12.1	11.5	11.9	13.0	13.7	13.7	14.6	16.1
360	4.4	4.0	4.2	4.6	14.3	14.4	15.2	15.0	20.7	21.8	21.4	22.1
480	2.9	4.4	4.9	5.5	8.5	13.7	16.0	15.0	13.8	20.5	25.3	22.6
600	3.3	4.2	3.9	5.0	11.9	14.7	13.9	17.8	25.5	25.4	23.8	27.7
720	3.0	4.0	4.3	5.6	10.8	15.1	16.3	16.8	17.8	26.0	25.3	26.7
840	6.0	2.3	4.2	3.7	15.0	13.5	14.5	14.6	23.5	20.4	20.4	22.1
960	6.4	4.5	5.9	4.5	15.6	15.5	14.6	14.7	22.1	22.1	24.3	22.0
1080	6.9	5.5	7.0	6.0	15.7	13.6	16.6	14.4	25.3	24.0	25.9	24.3
1200	4.1	3.8	4.7	4.7	11.9	12.3	12.8	11.8	18.7	19.7	20.1	18.1

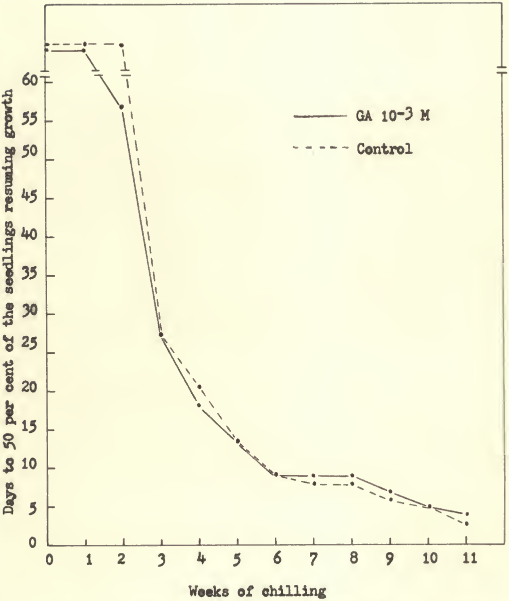


Figure 1. The influence of low temperature and gibberellic acid on the resumption of growth of dormant buds

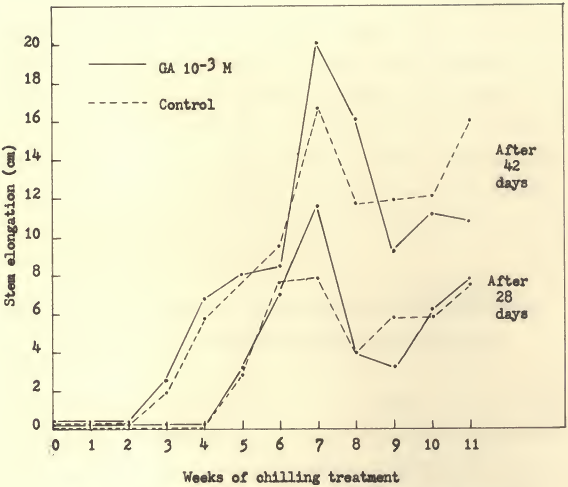


Figure 2. Effect of low temperature and gibberellic acid on stem elongation of seedlings at 28 and 42 days after treatment

It is noted that GA had little or no influence on the growth of the seedlings.

During the course of the foregoing observations, it was apparent that the degree of chilling had a marked influence on which buds resumed growth. This is shown graphically in Fig. 3. Low temperature treatments for a short duration (up to three weeks) resulted in an increased number of suckers (lateral buds breaking on any portion of the plant other than on the last flush of growth previous to dormancy). With further chilling (up to four weeks), the number of suckers decreased but the number of shoots (lateral buds breaking on the last flush of growth) increased. Any additional chilling beyond four weeks resulted in the reduction of both suckers and shoots. The application of GA under the conditions of this experiment did not alter these trends (Fig. 3).

Influence of low temperature, gibberellic acid, naphthaleneacetic acid, and maleic hydrazide on bud-break and stem elongation of Okinawa peach

For a foundation on which later experiments could be based, it was necessary to determine the responses of the Okinawa variety of peach to after-ripening at a low temperature and to the growth regulators to be used in this study. At the same time, the experiment was set up to investigate the possibility of an interaction between the thermo-removal of the block to growth by low temperature and three growth regulators, namely: GA, NAA and MH. To accomplish this, potted plants were first exposed for varying lengths of time to an after-ripening temperature before they were treated with GA, NAA or MH.

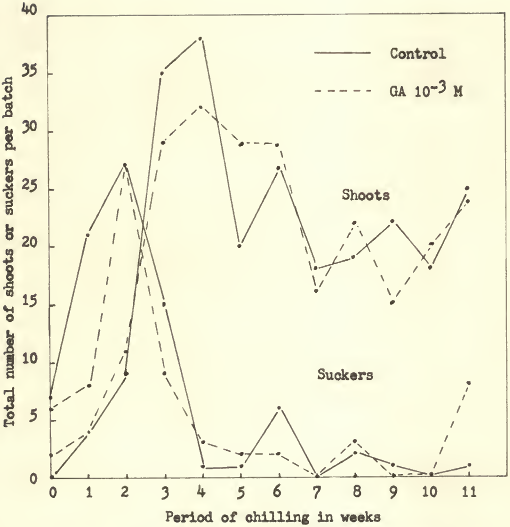


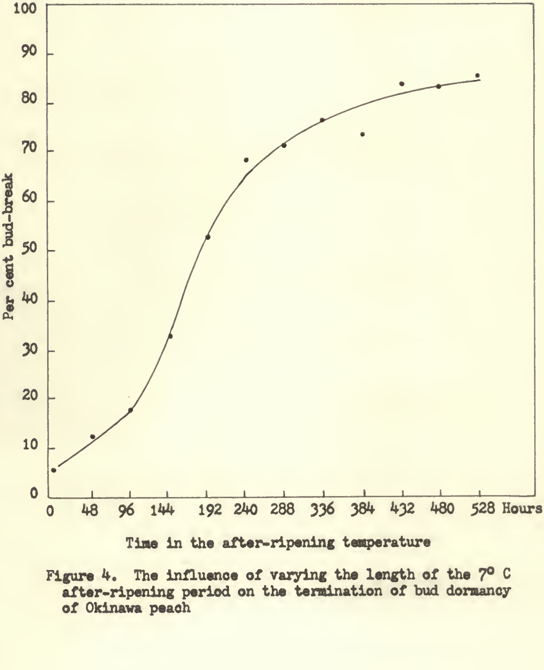
Figure 3. The influence of chilling and gibberellic acid on the number of shoots and suckers on seedlings

The bud-breaking response of Okinawa peach to an after-ripening temperature is shown graphically in Fig. 4. The 240 hours of pretreatment in a 7° C temperature caused a little over 50 per cent of the buds to break dormancy when the plants were returned to an environment favorable for growth.

In Table 3 the influence of chilling, GA, NAA, and MH on the termination of bud dormancy can be found. When the data were statistically analyzed, it was apparent that the effects of low temperature and chemicals, grouped collectively, were highly significant, but there was no significant influence from the different concentrations of any chemical nor was there any significant interaction between the growth regulators tested and the low temperature treatments.

The elongation of the main plant axis, as affected by chilling, GA, NAA and MH is given in Table 4. In general, it can be seen that the low temperature treatment increased the growth capacity of the plants, but that GA, NAA or MH in conjunction with different degrees of chilling did not seem to have any particular influence on stem elongation.

Following the foregoing preliminary test which showed a significant influence of the chemicals, additional experiments were designed to determine the influence of GA, NAA and MH on bud dormancy.



Time in the after-ripening temperature

Figure 4. The influence of varying the length of the 7° C after-ripening period on the termination of bud dormancy of Okinawa peach

TABLE 3

THE INFLUENCE OF LOW TEMPERATURE, GIBBERELLIC ACID, NAPHTHALENEACETIC ACID AND MALEIC HYDRAZIDE ON THE TERMINATION OF BUD DORMANCY[1]

Days of pretreatment at 7° ± 1° C	Per cent of buds broken at 20 days after treatment									
	Control	GA			NAA			MH		
		10^{-5}M	10^{-4}	10^{-3}	10^{-5}	10^{-4}	10^{-3}	10^{-5}	10^{-4}	10^{-3}
0	3.7	9.5	7.1	15.3	6.1	1.9	1.6	5.2	0.0	0.0
2	15.5	6.2	21.8	19.6	8.6	6.5	22.2	8.5	10.8	7.6
4	15.5	23.8	12.7	21.2	20.7	10.9	8.3	15.1	15.5	16.2
6	19.1	37.0	30.3	30.7	4.4	16.2	18.3	36.5	31.5	43.7
8	30.2	53.0	46.6	45.1	54.7	51.0	16.6	76.3	59.0	61.7
10	72.0	76.4	62.8	67.5	67.0	60.3	68.4	77.1	78.3	63.0
12	67.5	71.4	73.8	76.0	72.5	80.4	60.9	75.0	77.7	60.6
14	75.0	73.3	77.5	85.1	78.5	76.6	76.6	80.0	76.9	65.1
16	86.0	85.4	80.4	72.3	77.5	70.5	51.1	79.1	71.2	72.0
18	82.8	90.0	85.1	97.6	84.0	82.8	75.8	83.6	77.3	79.6
20	78.2	82.9	91.1	76.3	82.9	90.4	88.8	75.8	74.4	88.6
22	79.4	91.4	91.6	84.4	78.0	78.2	87.5	82.0	91.4	89.0

Source	D/F	SS	Mean square	F	
Pretreatment	11	430.8482	39.1680	106.1463	**
Concentration	2	0.3999	0.2000	---	N.S.
Chemical	2	5.8398	2.9199	7.9130	**
Interaction	92	33.9444	0.3690	---	N.S.

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[1] For detailed data see Tables 35, 36 and 37 (appendix).

TABLE 4

EFFECT OF CHILLING, GIBBERELLIC ACID, NAPHTHALENEACETIC ACID AND MALEIC HYDRAZIDE ON ELONGATION OF MAIN SHOOTS

No. of hours of chilling treatment	Average elongation in cm at 60 days after treatment									
	Control	GA			NAA			MH		
		10^{-5}M	10^{-4}	10^{-3}	10^{-5}	10^{-4}	10^{-3}	10^{-5}	10^{-4}	10^{-3}
0	0.1	0.9	0.0	1.3	0.4	0.4	0.5	1.0	1.4	0.5
48	1.9	1.5	1.6	2.2	1.7	1.7	1.4	0.1	0.7	0.9
96	1.8	1.0	1.5	0.4	3.4	2.9	3.7	2.0	1.2	0.5
144	1.2	1.8	1.7	2.7	2.1	1.0	3.4	2.7	2.0	3.5
192	2.8	3.6	5.5	5.8	3.9	4.9	3.4	3.8	2.7	2.6
240	6.0	8.1	4.4	2.8	5.0	5.7	5.6	4.7	4.2	7.0
288	5.6	4.7	7.7	6.4	7.5	5.7	7.5	5.8	5.6	5.9
336	8.8	8.2	7.1	6.0	7.0	8.1	7.5	6.8	6.3	6.9
384	7.0	6.5	6.8	6.7	7.0	7.4	5.2	7.6	6.7	8.1
432	6.6	9.3	8.5	10.3	10.5	8.1	7.3	10.2	9.1	8.9
480	8.8	6.8	8.5	12.8	8.1	7.9	10.7	7.8	10.6	11.0
528	12.6	12.7	14.4	14.8	13.2	14.8	12.5	14.1	11.8	10.3

The influence of gibberellic acid, naphthaleneacetic acid, and maleic hydrazide on bud dormancy

To test further the influence of GA, NAA, and MH on bud dormancy, the plants were pretreated at 7° C for 240 hours before the chemicals were applied. It was reasoned that partial chilling may poise the system controlling the block to growth so that it would be more sensitive to the agents added, especially compounds directly involved in dormancy.

The influence of GA and NAA, alone, and in various combinations on bud-break can be seen in Table 5. When the data were analyzed, the influence of NAA alone and in combination with GA was statistically highly significant. GA alone had no significant effect.

A graph of the influence of NAA on the termination of bud dormancy can be seen in Fig. 5. With low concentrations of NAA, a stimulation of bud-break occurred; with high concentrations, bud growth was inhibited. This promotion and inhibition effect on bud dormancy of NAA is similar to the influence of auxin on other growth and development phenomena.

The interaction between GA and NAA on bud-break is shown graphically in Fig. 6. In general, GA enhanced the activity of NAA. The promotion of bud-break was much greater in the presence of weak concentrations of GA. Also, the inhibition of bud-break was greater in the presence of relatively strong concentrations of GA.

The elongation of shoots as affected by GA and NAA is shown in Table 6. It appears that the GA at higher concentrations

TABLE 5

THE INFLUENCE OF GIBBERELLIC ACID AND NAPHTHALENEACETIC ACID ON BREAKING THE DORMANCY OF BUDS[1]

Concentration of GA	Per cent of buds broken at 13 days after treatment			
	Concentration of NAA			
	0	10^{-4}M	10^{-3}	10^{-2}
0	29.1	37.5	28.2	37.0
10^{-4}M	11.1	57.1	51.4	27.7
10^{-3}	34.2	43.7	36.6	19.2
10^{-2}	67.8	36.1	43.7	7.4

Source	D/F	SS	Chi square factor	Chi square
GA	3	0.2646	4.3735	1.1572 N.S.
NAA	3	2.7099	4.3735	11.8517 **
GA x NAA	9	8.1001	4.3735	35.5257 ***

[1] For detailed data see Table 38 (appendix).

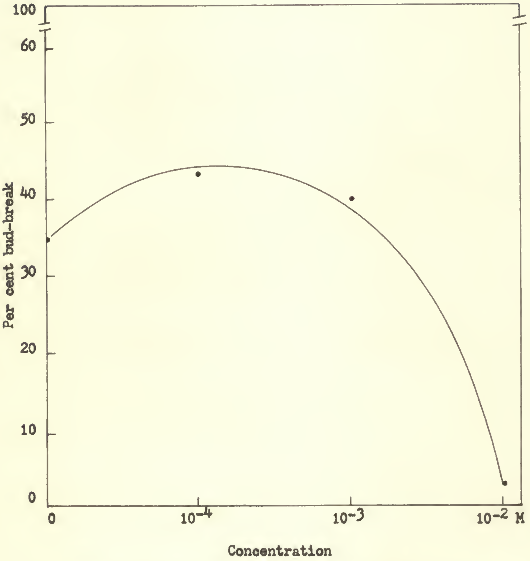

Figure 5. The influence of naphthaleneacetic acid on the termination of bud dormancy

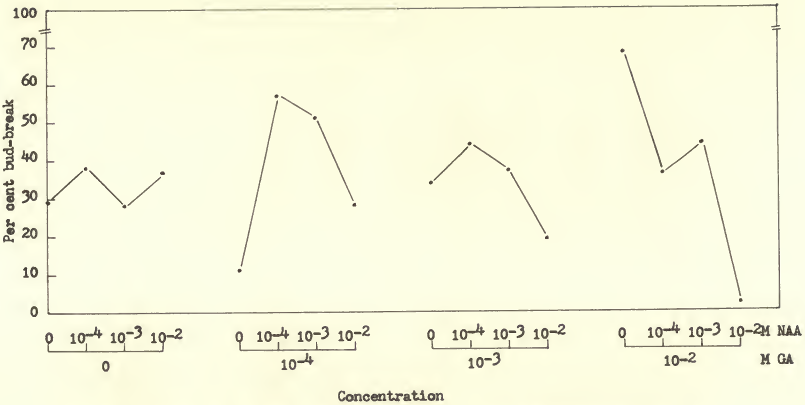

Figure 6. Interaction between gibberellic acid and naphthaleneacetic acid on the termination of bud dormancy

TABLE 6

INTERACTION BETWEEN CHILLING, GIBBERELLIC ACID AND NAPHTHALENEACETIC ACID ON ELONGATION OF MAIN SHOOTS

Concentration of GA	Average elongation in cm at 60 days after treatment			
	Concentration of NAA			
	0	10^{-4}M	10^{-3}	10^{-2}
0	7.4	7.0	6.4	6.3
10^{-4}M	6.6	6.5	3.2	6.1
10^{-3}	7.6	8.7	5.6	5.1
10^{-2}	12.7	10.4	9.1	9.6

increased the growth capacity of the buds. On the other hand, NAA inhibited the growth in the order of increasing concentration.

In the second experiment, GA and MH, alone, and in various combinations were tested for their influence on terminating the dormancy of peach buds. It appears from Table 7 that these chemicals had no significant influence on bud-break. However, GA was significant at $.30 > P .20 >$ level.

GA also caused an appreciable increase in stem elongation as can be seen in Table 8. MH had little or no influence on stem elongation.

In the third test, the influences of NAA and MH, alone, and in various combinations on bud-break was investigated. It can be seen in Table 9 that NAA alone or in mixtures with MH had no influence on bud-break. However, MH promoted bud-break at 10^{-4} M and 10^{-2} M concentrations but inhibited at 10^{-3} M concentration. A suggestion for this type of action by MH can not be made on the basis of these experiments.

The action of NAA and MH on stem elongation is shown in Table 10. There seems to be no significant effect of NAA or MH on stem elongation.

The influence of gibberellic acid and indole-3-acetic acid on the termination of bud dormancy

Since the foregoing experiments indicated a possible interaction between GA and an auxin, additional investigations were conducted to examine further this action of growth regulators on the termination of bud dormancy. Three changes were made in the

TABLE 7

THE INFLUENCE OF GIBBERELLIC ACID AND MALEIC HYDRAZIDE ON BREAKING THE DORMANCY OF BUDS[1]

Concentration of GA	Per cent of buds broken at 13 days after treatment			
	Concentration of MH			
	0	10^{-4}M	10^{-3}	10^{-2}
0	29.1	44.4	9.3	50.0
10^{-4}M	39.2	32.5	39.2	38.7
10^{-3}	36.1	36.1	44.1	31.4
10^{-2}	41.9	48.4	37.5	53.5

Source	D/F	SS	Chi square factor	Chi square	
GA	3	0.9505	4.3837	4.1667	N.S.
MH	3	0.7447	4.3837	3.2645	N.S.
GA x MH	9	3.2203	4.3837	14.1168	N.S.

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[1] For detailed data see Table 39 (appendix).

TABLE 8

INTERACTION BETWEEN CHILLING, GIBBERELLIC ACID AND MALEIC HYDRAZIDE ON ELONGATION OF MAIN SHOOTS

Concentration of GA	Average elongation in cm at 60 days after treatment			
	Concentration of MH			
	0	10^{-4}M	10^{-3}	10^{-2}
0	7.4	9.5	7.8	5.8
10^{-4}M	7.4	7.1	8.0	5.7
10^{-3}	9.2	8.4	8.3	7.3
10^{-2}	12.8	13.8	15.1	11.5

TABLE 9

THE INFLUENCE OF NAPHTHALENEACETIC ACID AND MALEIC HYDRAZIDE ON BREAKING THE DORMANCY OF BUDS[1]

Concentration of NAA	Per cent of buds broken at 13 days after treatment			
	Concentration of MH			
	0	10^{-4}M	10^{-3}	10^{-2}
0	29.1	44.4	9.3	50.0
10^{-4}M	37.5	43.7	17.1	20.0
10^{-3}	28.2	28.4	28.1	18.7
10^{-2}	37.0	30.0	12.4	37.5

Source	D/F	SS	Chi square factor	Chi square	
NAA	3	1.3017	4.7721	6.2118	N.S.
MH	3	3.4280	4.7721	16.3587	***
NAA x MH	9	1.6302	4.7721	7.7794	N.S.

[1] For detailed data see Table 40 (appendix).

TABLE 10

INTERACTION BETWEEN CHILLING, NAPHTHALENEACETIC ACID AND MALEIC HYDRAZIDE ON THE ELONGATION OF THE MAIN SHOOTS

Concentration of NAA	Average elongation in cm at 60 days from treatment			
	Concentration of MH			
	0	10^{-4}M	10^{-3}	10^{-2}
0	7.4	9.5	7.8	5.8
10^{-4}M	7.0	6.3	7.7	3.4
10^{-3}	6.4	7.3	7.6	6.3
10^{-2}	6.3	9.1	4.3	11.5

techniques. First, the dormant plants received no chilling prior to treatment with the chemicals; second, IAA was used in the tests instead of NAA; and third, the chemicals were applied by injecting the test solution directly inside the bud scales onto the apex of the bud. As can be seen in Table 11, both IAA and GA, alone, and in various combinations had a highly significant influence on bud dormancy. Ga accelerated bud-break as the concentration increased. IAA had the promotion and inhibition type of curve that resembled the action exhibited by NAA in an earlier experiment. A 10^{-4} M concentration promoted bud-break while a 10^{-3} M concentration had a lesser effect on overcoming bud dormancy. The influence of IAA and GA on bud dormancy seems to be additive. A further graphic representation of these data can be seen in Fig. 7. The shape of the action curves for the chemicals are quite different. With the strongest concentration of GA used, there was no inhibition of bud-break. This is in contrast to the action of IAA.

Since these data indicate that growth regulators have an influence on bud dormancy and that the action of an auxin has a "two-phase" characteristic, it would seem reasonable to suggest that bud dormancy is controlled in part by hormones.

Discussion

The majority of the evidence obtained from these experiments indicates that GA and auxin have a marked influence on peach bud dormancy.

TABLE 11

THE INFLUENCE OF GIBBERELLIC ACID AND INDOLE-3-ACETIC ACID ON BREAKING THE DORMANCY OF BUDS[1]

Concentration	Per cent of buds broken at 14 days after treatment
Control	3.6
GA 10^{-6}M	25.9
GA 10^{-5}	29.1
GA 10^{-4}	37.7
GA 10^{-3}	42.8
IAA 10^{-6}	10.0
IAA 10^{-5}	16.0
IAA 10^{-4}	32.4
IAA 10^{-3}	21.7
GA 10^{-4} + IAA 10^{-6}	60.4
GA 10^{-4} + IAA 10^{-5}	62.8
GA 10^{-4} + IAA 10^{-4}	27.4
GA 10^{-4} + IAA 10^{-3}	25.0

Source	D/F	SS	Chi square factor	Chi square	
Concentration	3	2.2473	5.0470	11.3421	**
Chemical	1	2.0931	5.0470	10.5638	**
Interaction	3	4.4346	4.7472	21.0519	***

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[1] For detailed data see Table 41 (appendix).

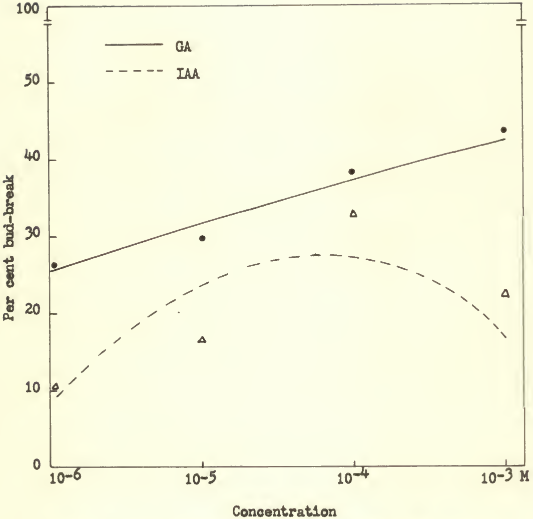

Figure 7. The influence of gibberellic acid and indole-3-acetic acid on breaking the dormancy of buds

In the case of the preliminary experiments with the variety Lovell, GA was effective in causing bud-break and shoot elongation only where it was applied to seedlings which had received very little chilling. It was thought at the time that one of the reasons for the small influence on bud-break might be that a higher concentration was needed. Therefore, in later experiments higher concentrations (10^{-2} M) were used.

Further experiments on GA reveal that it was effective in breaking dormancy of bud, but not to the extent expected. In all of these experiments, GA was incorporated into lanolin paste and applied on the side of the stem 5 cm from the apical bud. Therefore, one reason for the weak response to GA could be that the chemical did not get to the site of action. This explanation was supported by the results of an additional experiment. When GA was applied to the plants by injecting the test solution inside the bud scales onto the apical growing region, it was highly significant in terminating the dormancy of peach buds.

These results on GA are consistent with others who have reported an influence of GA on bud dormancy. Donoho and Walker (28, 87) stated that they were able to break the dormancy of peach buds by the application of GA. Barton and Chandler (6) also broke the epicotyl dormancy of the peony tree by the use of GA, and Brown, et al. (19), working on pear, reported a breaking of dormancy by GA with trees which had received partial chilling.

The influence of NAA on bud dormancy was inconsistent. While in one experiment NAA, regardless of concentration, increased the number of buds breaking dormancy; in another, low concentrations promoted bud growth and high concentrations inhibited; and in another, there were no significant effects from NAA application. Since in these experiments the growth regulator was also applied in lanolin, one reason for variation in the results may have been caused by different amounts of NAA getting to the site of action. In an additional experiment, another auxin (IAA) was injected inside the bud scales onto the apex. This type of treatment proved to be highly effective in causing buds to resume growth. Low concentrations promoted bud-break but high concentrations inhibited. This evidence would seem to indicate that the dormancy of buds may be in part controlled by auxins. This is in line with the work of Zimmerman (95) who could ascertain auxin only in sprouting buds, whereas Avery, et al. (3) could find no auxin in dormant buds. Moreover, Amlong and Naundrop (2), by applying IAA and other compounds directly to the buds, obtained a forcing action. Furthermore, Biggs (7) and Blommaert (9) found growth promotors in non-dormant peach buds that were not present in dormant buds.

It was observed that NAA inhibited bud-break and elongation at high concentrations. Similar results were obtained by Sell, et al. (78, 79) who were able to prolong the dormancy of buds of tung by the repeated applications of high doses of NAA and IAA. Since auxins had a two-phased action, it is possible that dormancy can be controlled in part by either a sub- or super-optimal level of phytohormone in the tissue.

An interaction between GA and NAA was observed on bud-break. A mixture of low concentrations of the two chemicals was effective in causing buds to resume growth. This influence of a mixture of the two chemicals on bud-break was greater than the sum of the effects of the two chemicals alone. An interaction between GA and IAA with about the same trend was also obtained. There are other reports in the literature that the influences of GA and auxin on growth are more than additive (37).

It was observed that chilling was very effective in breaking dormancy. It appeared that insufficient chilling caused the seedlings to produce suckers in larger numbers. However, the proper amount of chilling resulted in decreasing the number of suckers and increasing the growth of lateral buds on the last flush of growth that occurred previous to bud dormancy. An excessive amount of chilling caused fewer shoots and poorer growth on the buds breaking dormancy.

Although GA and auxins have a marked influence on the dormancy of peach buds, it would seem reasonable to conclude that they are not the primary agents controlling dormancy. This is based mainly on the observation that low temperature is a much more effective inducer of bud growth than synthetic hormones tested to date. It could be that hormones may become the limiting factor in certain phases of dormancy after the initial block to growth is removed.

Summary

To gain further knowledge of the role of growth regulators in controlling bud dormancy of woody plants, a study was made of the possible interactions between an after-ripening temperature and GA, NAA, IAA and MH on the rest period of peach buds. The following results were obtained:

1. GA effectively broke bud dormancy on non-chilled and partially chilled peach plants.

2. NAA and IAA promoted bud-break at low concentrations and inhibited bud-break at high concentrations.

3. MH had little or no influence on the dormancy of peach buds.

4. There was a definite interaction between GA and auxin on the breaking of bud dormancy.

5. Low temperatures were more effective in terminating bud dormancy than GA, NAA, IAA or MH.

The duration of the low-temperature treatment determined the pattern of bud-break on a plant. Insufficient chilling caused buds at the base of the plant to resume growth. Excessively long exposures to low temperatures caused terminal buds to break without many lateral buds resuming growth. Intermediate between these two extremes was a balance of terminal and lateral buds resuming growth.

THE INFLUENCE OF GIBBERELLIC ACID, NAPHTHALENEACETIC ACID AND MALEIC HYDRAZIDE ON SEED DORMANCY

Introduction

Rest or dormant periods are common in the plant kingdom and have been studied extensively in such organs as buds, corms, tubers and seeds. The latter organs provide particularly favorable material for investigating dormancy since they can be considered a closed system, at least to some extent.

Most seeds contain all the essential elements for germination except water. Yet, the seeds of many plants will not germinate after they have been allowed to imbibe water in an environment favorable for growth and development. Seeds responding in this manner are in a state of dormancy or rest (85). Crocker (27) described six classes of seed dormancy, ranging from a simple mechanical restriction of transport or expansion to a non-mechanical block to growth intrinsic to the seed. Peach seeds have the latter type of rest period.

As has been described in detail in a foregoing section (pages 3 to 19), previous investigations indicate that the rest period of certain seeds, which is caused by internal factors, may be hormonally controlled. It was the purpose of this study to test this concept. This was attempted by observing the response of the organism to exogenously applied growth regulators.

Materials and Methods

To investigate the relation of hormones to seed dormancy the following experiments were conducted:

1. The influence of gibberellic acid on peach seed dormancy

The seeds of *Prunus persica* var. Lovell were soaked in various concentrations of gibberellic acid (GA) for 16 hours and then planted in flats of vermiculite. Twenty seeds per treatment were used. Data were taken on seed germination.

2. (a) Interaction between gibberellic acid and naphthaleneacetic acid
   (b) Interaction between gibberellic acid and maleic hydrazide
   (c) Interaction between naphthaleneacetic acid and maleic hydrazide

The seeds of peach var. Lovell were used. The seeds were treated by soaking them for eight hours in 10 ml of distilled water with or without addendum. After soaking, the seeds were blotted and planted in 4-inch plastic pots. The experiment was designed so that the data taken on seed germination could be statistically analyzed by the "factorial chi square" method.

In another experiment the seeds were handled identically to the method outlined above (2) except that they were given a chilling pretreatment of 1000 hours at $7^{\circ} \pm 1^{\circ}$ C before the treatment with chemicals.

Experimental Results

The influence of gibberellic acid, naphthaleneacetic acid and maleic hydrazide on the germination of non-chilled peach seeds

A preliminary experiment was conducted to test the influence of various concentrations of GA on overcoming the dormancy of seeds. As can be seen in Table 12, higher concentrations of GA (10^{-3} and 10^{-2} M) caused 100 per cent germination, whereas low concentrations of GA had no visible effect on germination.

After noting that certain concentrations of GA would stimulate peach seed germination, further detailed experiments were conducted. In these experiments GA (10^{-6} to 10^{-2} M), naphthaleneacetic acid (NAA) (10^{-6} to 10^{-3} M) and maleic hydrazide (MH) (10^{-6} to 10^{-3} M), alone, and in various combinations, were applied to non-chilled peach seeds by soaking them for eight hours. The purpose was to determine if there was an interaction between GA, auxin and an auxin antagonist on peach seed germination.

The effect of GA and NAA on seed germination is shown in Table 13. It appears that both GA and NAA alone had a significant influence on seed germination. However, interactions between these compounds on seed germination were not significant. It should be further observed from Fig. 8 that GA stimulated maximum germination at a concentration of 10^{-2} M. NAA inhibited germination as the concentration was increased until there was no germination with 10^{-3} M.

Seed germination as affected by GA and MH is presented in Table 14. No statistical significant effect on germination was caused by MH alone, or in combinations with GA. However, GA was significant at $.20 > P .10 >$ level.

TABLE 12

THE NUMBER OF SEEDS GERMINATING AFTER TREATMENT WITH GIBBERELLIC ACID

Concentration of GA	Per cent germinated after 3 weeks
0	0
10^{-6} M	0
10^{-5}	0
10^{-4}	0
10^{-3}	100
10^{-2}	100

TABLE 13

THE NUMBER OF SEEDS GERMINATING AFTER TREATMENT WITH GIBBERELLIC ACID AND NAPHTHALENEACETIC ACID[1]
(20 seeds per treatment)

Concentration of GA	Concentration of NAA					Total No. of seeds germinated
	0	10^{-6}M	10^{-5}	10^{-4}	10^{-3}	
0	1	2	1	1	0	5
10^{-6}M	7	5	4	2	0	18
10^{-5}	4	4	5	3	0	16
10^{-4}	3	3	1	2	0	9
10^{-3}	8	3	4	2	0	17
10^{-2}	2	7	6	5	0	20
Total No. of seeds germinated	25	24	21	15	0	85

Source	D/F	SS	Chi square factor	Chi square	
GA	5	1.7083	8.2239	14.0488	*
NAA	4	3.5166	8.2239	28.9201	**
GA x NAA	20	2.5834	8.2239	21.2456	N.S.

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[1] For detailed data see Table 42 (appendix).

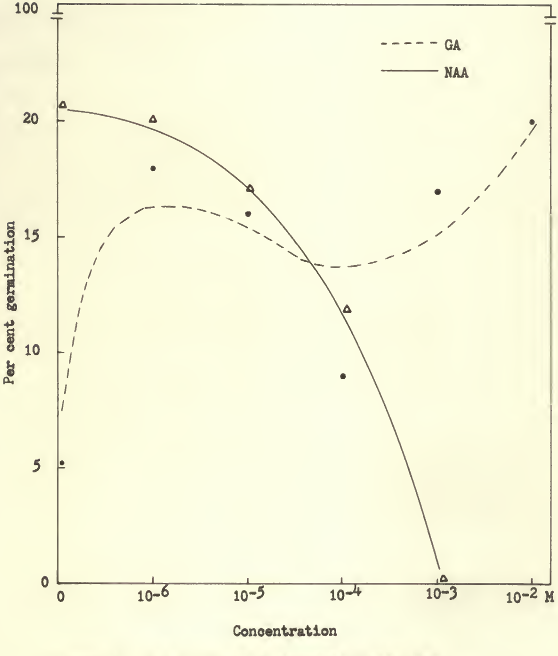

Figure 8. The influence of gibberellic acid and naphthaleneacetic acid on the germination of seeds

TABLE 14

THE NUMBER OF SEEDS GERMINATING AFTER TREATMENT WITH GIBBERELLIC ACID AND MALEIC HYDRAZIDE[1]
(20 seeds per treatment)

Concentration of GA	Concentration of MH					Total No. of seeds germinated
	0	10^{-6}M	10^{-5}	10^{-4}	10^{-3}	
0	4	6	2	2	2	16
10^{-6}M	1	3	2	2	6	14
10^{-5}	2	6	1	7	5	21
10^{-4}	3	4	3	4	2	16
10^{-3}	1	6	6	3	1	17
10^{-2}	8	4	6	5	5	28
Total No. of seeds germinated	19	29	20	23	21	112

Source	D/F	SS	Chi square factor	Chi square	
GA	5	1.3133	6.5866	8.6501	N.S.
MH	4	0.5265	6.5866	0.3467	N.S.
GA x MH	20	4.0935	6.5866	26.9622	N.S.

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[1] For detailed data see Table 43 (appendix).

The influence of NAA and MH on seed germination is shown in Table 15. It should be noted that NAA alone affected seed germination, but MH, alone, or the combinations of the two chemicals, had no effect on germination. A graph of the data (Fig. 9) further reveals that NAA in low concentrations (10^{-6} to 10^{-4} M) resulted in the promotion of seed germination, but stronger concentrations inhibited germination.

The following points are evident from the data presented above:

1. GA promoted germination in the higher concentrations.

2. The influence of NAA was highly significant in both experiments. It promoted germination in low concentrations and inhibited germination in high concentrations.

3. MH in the concentrations used had no statistical effects on peach seed germination.

4. Under the conditions of these experiments, no significant interaction between the chemicals could be detected.

The influence of gibberellic acid, naphthaleneacetic acid and maleic hydrazide on the germination of partially chilled peach seeds

From previous work in this laboratory (Biggs, unpublished), it was noted that with a given population of Lovell peach seeds receiving 1000 hours of an after-ripening temperature (4° to 8° C) there would be 30 to 80 per cent of the seeds germinating. In an attempt to poise the system, seeds were chilled for 1000 hours before being used in the following experiments.

The influence of GA and NAA on the germination of peach seeds can be seen in Table 16. The data show that the germination induced by GA alone was statistically significant. The treatment

TABLE 15

THE NUMBER OF SEEDS GERMINATING AFTER TREATMENT WITH NAPHTHALENEACETIC ACID AND MALEIC HYDRAZIDE[1] (20 seeds per treatment)

Concentration of NAA	Concentration of MH					Total No. of seeds germinated
	0	10^{-6}M	10^{-5}	10^{-4}	10^{-3}	
0	3	0	0	3	0	6
10^{-6} M	1	4	2	1	2	10
10^{-5}	0	3	4	2	4	13
10^{-4}	3	5	2	5	3	18
10^{-3}	0	0	0	0	0	0
10^{-2}	0	0	0	0	0	0
Total No. of seeds germinated	7	12	8	11	9	47

Source	D/F	SS	Chi square factor	Chi square	
NAA	5	2.6084	13.8509	36.1286	**
MH	4	0.1433	13.8509	1.9848	N.S.
NAA x MH	20	1.6167	13.8509	22.3937	N.S.

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[1] For detailed data see Table 44 (appendix).

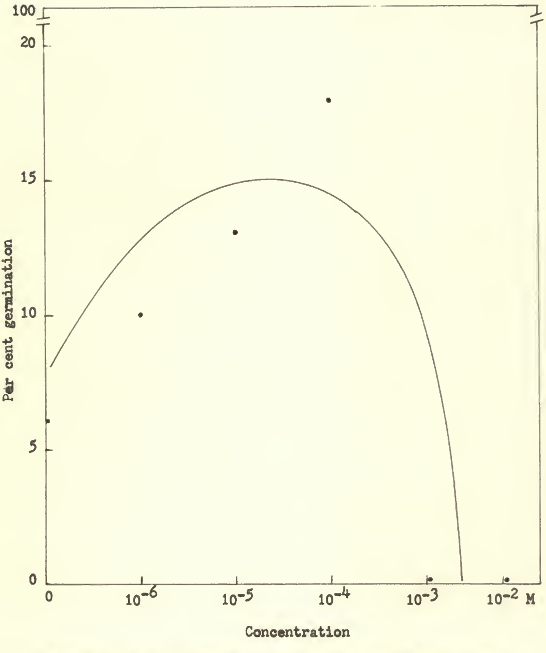

Figure 9. The influence of naphthaleneacetic acid on the germination of seeds

TABLE 16

EFFECT OF GIBBERELLIC ACID AND NAPHTHALENEACETIC ACID ON THE NUMBER OF SEEDS GERMINATING AFTER AFTER-RIPENING AT 7° C FOR 1000 HOURS[1]

(20 seeds per treatment)

Concentration of GA	Concentration of NAA					Total No. of seeds germinated
	0	10^{-6}M	10^{-5}	10^{-4}	10^{-3}	
0	0	6	6	4	0	16
10^{-6} M	1	4	2	2	0	9
10^{-5}	4	2	2	5	0	13
10^{-4}	3	4	6	3	0	16
10^{-3}	3	6	5	3	1	18
10^{-2}	4	11	5	2	2	24
Total No. of seeds germinated	15	33	26	19	3	96

Source	D/F	SS	Chi square factor	Chi square	
GA	5	1.2600	7.4404	9.3749	*
NAA	4	4.3066	7.4404	32.0428	**
GA x NAA	20	3.1734	7.4404	23.6113	N.S.

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[1] For detailed data see Table 45 (appendix).

of NAA alone was highly significant. It should be observed in Fig. 10 that the maximum seed germination was obtained by the concentrations of 10^{-6} M of NAA. Other concentrations decreased the germination in the order of increasing concentrations.

The influence of GA and MH on seed germination can be seen in Table 17. It appears that MH alone has no effect on seed germination. GA promoted seed germination at the statistical level of $.10 > P .05 >$. It appears from Table 17 that there is a significant interaction between GA and MH, no definite conclusions can be drawn from the data since neither of the chemicals alone had a significant effect on germination.

The influence of NAA and MH on the germination of seeds can be found in Table 18. The results indicate that NAA alone had a significant effect on seed germination, MH had no effect. Fig. 11 reveals that NAA caused promotion of seed germination with low concentrations but inhibited the higher concentrations of 10^{-3} and 10^{-2} M.

Discussion

GA was effective in inducing the growth of dormant peach seeds. Although in certain experiments the influence of GA was not statistically significant, it was apparent from an inspection of the data that it had a promoting effect on germination in each test. Since low concentration of GA and short times of exposures of peach seeds to GA were chosen so that it would be possible to obtain interactions with auxins and auxin antagonists, the quantity of GA needed at the site of action for a larger response was not realized.

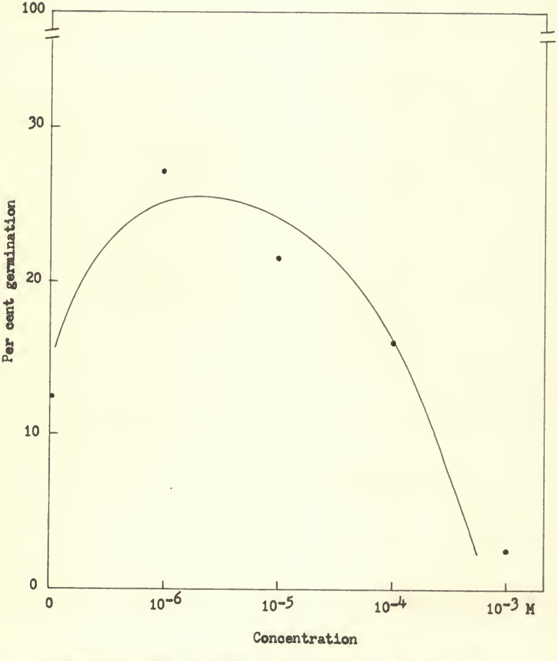

Figure 10. The influence of naphthaleneacetic acid on the germination of seeds after-ripened 1000 hours at 7° C temperature before chemical treatment

TABLE 17

EFFECT OF GIBBERELLIC ACID AND MALEIC HYDRAZIDE ON THE NUMBER OF SEEDS GERMINATING AFTER AFTER-RIPENING AT 7° C AT 1000 HOURS[1]

(20 seeds per treatment)

Concentration of GA	Concentration of MH					Total No. of seeds germinated
	0	10^{-6}M	10^{-5}	10^{-4}	10^{-3}	
0	7	14	13	12	9	55
10^{-6}M	10	15	16	14	10	65
10^{-5}	17	16	12	12	13	70
10^{-4}	17	2	13	13	11	56
10^{-3}	15	14	12	15	12	68
10^{-2}	16	14	14	11	15	70
Total No. of seeds germinated	82	75	80	77	70	384

Source	D/F	SS	Chi square factor	Chi square	
GA	5	2.1667	4.3402	9.4039	*
MH	4	0.5499	4.3402	0.2386	N.S.
GA x MH	20	11.2501	4.3402	48.8276	**

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[1] For detailed data see Table 46 (appendix)

TABLE 18

EFFECT OF NAPHTHALENEACETIC ACID AND MALEIC HYDRAZIDE ON THE NUMBER OF SEEDS GERMINATING AFTER AFTER-RIPENING AT 7° C FOR 1000 HOURS[1]

(20 seeds per treatment)

Concentration of NAA	Concentration of MH					Total No. of seeds germinated
	0	10^{-6}M	10^{-5}	10^{-4}	10^{-3}	
0	7	13	12	14	12	58
10^{-6}M	12	13	12	13	11	61
10^{-5}	13	15	11	13	15	67
10^{-4}	13	15	14	12	14	68
10^{-3}	6	4	1	3	6	20
10^{-2}	0	0	0	0	0	0
Total No. of seeds germinated	51	60	50	55	58	274

Source	D/F	SS	Chi square factor	Chi square	
NAA	5	40.8534	4.0302	164.6473	**
MH	4	0.6233	4.0302	2.5120	N.S.
NAA x MH	20	2.6967	4.0302	10.8682	N.S.

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[1] For detailed data see Table 47 (appendix).

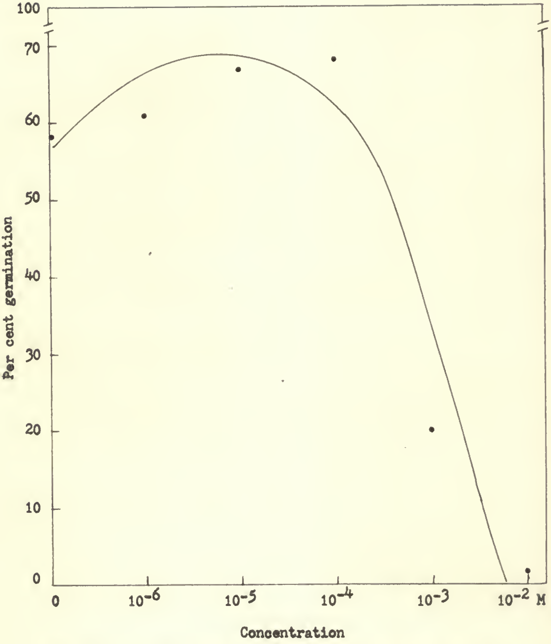

Figure 11. The influence of naphthaleneacetic acid on the germination of seeds after-ripened at 7° C prior to chemical treatment

The growth response of peach seeds and seeds of other species of Prunus to gibberellins have been reported previously. Donoho and Walker (28) reported that the dormancy of peach seeds was overcome by GA. Also, Fogle (35) observed that GA was able to substitute in part for the after-ripening of cherry seeds by low temperature, but the latter was a more effective dormancy breaking treatment.

The influence of GA on peach seed germination seems to be independent of the effect of chilling since both non-chilled and chilled seeds responded the same. This influence of GA on peach seed germination may be similar to the effects of the chemical on germination of seeds which do not require after-ripening.

Hayashi (41) demonstrated that gibberellins accelerated the germination of barley, wheat and rice grains. Ching and Stewart (21) reported that the seeds of papaya, orchids, and various other varieties of native Californian annuals responded to GA treatment. Gibberellins also promoted the sprouting of many weed seeds (21), wild oats (43), beans, peas and sweet corn (92).

The most pronounced and persistent observation of this work was that the germination of peach seeds was promoted by the low concentration of NAA (10^{-6}, 10^{-5}, and 10^{-4} M). This was true with chilled as well as unchilled seeds. This effect of an auxin on germination has been reported once previously. Barton (4) found an increase in the germination of seeds of American elm by the application of NAA (3.7 to 35.5 mg/liter). In this same report she found no effect of low concentration of IAA or NAA on the germination of apple seeds.

In view of the nature of this response of peach seeds to NAA, it would seem that auxins are not the primary factors controlling dormancy. This statement is based primarily on the observation that the promotion of seed germination by auxin is not as effective as after-ripening at low temperatures. However, there was a response of promotion of seed germination by NAA. This could indicate that an optimum concentration of auxin is beneficial for germination. If the block to growth is caused by a delicately balanced biological system, which would seem to be the case, then any agent which would have a positive influence on growth could promote some seeds to germinate.

It is possible that chemical inhibitors of growth are controlling dormancy and that hormones are blocked in their action until the inhibitors have decreased to a certain level. Blumenthal-Goldschmidt (10) observed that with the breaking of dormancy of lettuce seeds the amount of growth inhibitors decreased and growth promotors increased. Also, Yoshidu (94) reported that with the increase in chilling treatment of seeds of *Abies sachalinesis* the growth promotors increased and growth inhibitors decreased.

It was observed that high concentrations of NAA inhibited germination of chilled as well as unchilled seeds. This was also observed by Barton (4) who found that NAA in high concentration (106.6 and 320.0 mg/liter) inhibited the germination of the seeds of American elm. She also reported that IAA and NAA (10 to 100 mg/liter) inhibited the germination of after-ripened seeds of dogwood and purple crabapple. Furthermore, Everson and Dunham (33) found that a high concentration (10^{-3} M) of 2,4-dichlorophenoxyacetic acid inhibited the germination of seeds of wheat, beans and other crops. Thus it seems that high concentration of auxins can inhibit the germination of seeds. However, dormancy of seeds in nature is not due to a super-optimal concentration of auxin or other growth promoting substances since seed germination can be stimulated by low concentrations of auxin.

MH did not affect seed germination. Similar results were obtained by Haber and White (40), who did not find an inhibiting effect of MH at concentrations from 10^{-7} to 10^{-3} M.

The present results indicate no interaction between chilling, GA, NAA and MH on seed germination, except for the significant interaction between GA and MH that was shown in Table 17. Since neither of these chemicals alone had a significant effect on germination, no definite reason for this action can be given at this time.

The results from this investigation indicate that GA and auxin do not seem to be involved directly in natural dormancy. Both substances have a positive influence on seed germination but it was not additive to the influences of low temperatures on germination.

Summary

1. To investigate further the concept that dormancy may be hormonally controlled, the influence of various combinations of GA, NAA and MH on seed germination was tested.

2. GA induced seed germination in both chilled and unchilled seeds.

3. Regardless of chilling treatments, low concentrations of NAA promoted and high concentrations inhibited seed germination.

4. MH had no significant effect on seed germination.

5. In determining if any significant interactions were observed between chilling, GA, NAA and MH on seed germination, only a statistically significant interaction was observed between GA and MH.

THE INFLUENCE OF ANAEROBIOSIS AND A BIOLOGICALLY HIGH TEMPERATURE ON BUD AND SEED DORMANCY

Introduction

Since anaerobic conditions and a biologically high temperature have been found to reverse the vernalization of plant organs (22), it would seem reasonable to determine if the thermo-sensitive processes of bud and seed dormancy are reversible. Although vernalization of plant organs involves the qualitative response of floral initiation, and the thermo-removal of the rest period of plant organs involves a quantitative response of the rate of growth, there are similarities that exist between the two phenomena, e.g., the temperature of the reaction is approximately the same, the same degree of imbibition of organs is needed before the system will respond, etc. (22).

To investigate the possibility of the reversibility of the low temperature induction phase of growth both peach seeds and buds were used.

Materials and Methods

The influence of high temperatures on the possible reversal of the chilling treatment of seeds

Two hundred seeds of peach var. Lovell were planted in flats of perolite and placed in a refrigerator at 7° ± 1° C for 1000, 1300 or 1600 hours. Immediately after the 7° ± 1° C pretreatments, the seeds were exposed to 0° ± 1° C, 26° ± 1° C, 37° ± 1° C and 46° ± 1° C for eight hours. This treatment was provided by placing the seeds just beneath the surface of a distilled water bath, kept at the designated temperature. Following this treatment, the seeds were planted in 4-inch plastic pots in perolite. Data on seed germination and seedling height were recorded at regular intervals.

Later, additional experiments were conducted in order to determine the physical conditions of the seeds following the high temperature treatments. To accomplish this, seeds were exposed to 7° ± 1° C for 1000 hours before being subjected to 0° ± 1° C, 26° ± 1° C, 37° ± 1° C, and 46° ± 1° C. Out of 40 seeds used per treatment, 20 seeds were planted immediately in 4-inch plastic pots in vermiculite. The remaining 20 seeds were again exposed to 7° ± 1° C for 500 hours. After this treatment, the seed coats of half of the seeds of each treatment were removed and all the seeds planted immediately. Data were taken on seed germination.

To investigate the relation of temperature following thermo-removal of the block to growth on promoting or inhibiting germination, seeds were exposed to 7° ± 1° C for 1000 hours before being placed under different temperature regimes for varying lengths of time. Immediately after this treatment the seeds were sown in 4-inch plastic pots in perolite. Data were recorded on seed germination.

The influence of nitrogen on the possible reversal of the chilling treatment of seeds

Six hundred seeds of peach var. Lovell were placed in a $7^{\circ} \pm 1^{\circ}$ C refrigerator for each pretreatment of 1000, 1300 or 1600 hours. Of these, 400 seeds were placed immediately in an oxygen-free environment for eight hours. The treatment was given by submerging the seeds in oxygen-free water and continuously bubbling nitrogen gas through it. Half of the treated along with 200 non-treated seeds were sown immediately. The remaining 200 nitrogen-treated seeds were given a rechilling treatment of $7^{\circ} \pm 1^{\circ}$ C for 1000 hours before they were planted.

The influence of high temperature and nitrogen on the possible reversal of the chilling treatment of buds

For these experiments, rooted cuttings of *Prunus persica* var. Okinawa were used. The dormant plants were given a chilling pretreatment of 24 hours at $7^{\circ} \pm 1^{\circ}$ C before they were placed in high temperatures or in an oxygen-free atmosphere for eight hours. The high temperature treatments were given by placing the test material in incubators fixed at the designated temperatures. The nitrogen treatment was provided by enclosing the cuttings into a plastic bag and continuously passing nitrogen gas through it. Twenty cuttings per treatment were placed in a shade house for growing and the other half were given an additional chilling treatment of 240 hours before they were placed in the shade house. Data were recorded on bud-breaks and elongation.

Experimental Results

The influence of high temperature on the reversal of the chilling treatment of seeds

The influence of high temperatures on the possible reversal of the chilling treatment of seeds was investigated. It is apparent from Fig. 12 that a 26° C temperature has no appreciable effect on peach seed germination, but the treatment of 37° C drastically reduced the number of seedlings emerging from the soil. A comparison of this data with that presented in Table 19 will reveal that the 37° C temperature may have slightly influenced seed germination, but the effect was a lessening of the growth capacity of the seedlings. (A measure of the growth capacity of the sprouted seeds was rate of growth.) The seeds sprouted but there was little root or shoot elongation of the seedlings. Further evidence that high temperatures decreased the growth capacity of the seedlings can be seen in Table 20. Increasing the temperature decreased the height of the seedlings. The 46° C treatment failed to stimulate germination and 86 per cent of the seeds rotted (Table 19).

The influence on this phenomena of increasing the chilling pretreatment from 1000 to 1300 hours was also investigated. It is evident from Fig. 13 that an increase in the chilling pretreatment caused the seeds to respond in a manner similar to the 1000 hours pretreatment. However, there were a few quantitative differences. The rate of seed germination in the control and the 26° C treatment was more rapid. Also, the 37° C temperature was more effective in reducing the growth capacity of the seedlings, as illustrated in

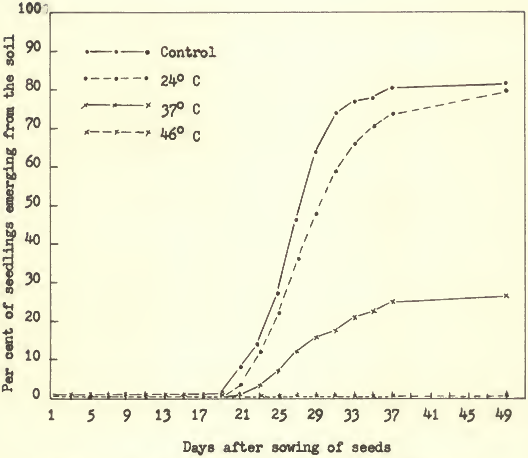

Figure 12. Effect of high temperature, following 1000 hours after-ripening at 7° C, on seed germination

TABLE 19

THE INFLUENCE OF VARIOUS TEMPERATURES ON GERMINATION, GROWTH AND VIABILITY OF SEEDS WHICH HAD BEEN PREVIOUSLY AFTER-RIPENED FOR 1000 HOURS AT 7° C

Temperature treatment for 8 hours following after-ripening	Per cent of seeds germinated		Per cent of seeds not germinated	
	Shoots emerged from soil	Shoots not emerged from soil	Viable	Rotted
Control (planted immediately)	81.5	6.0	1.5	11.0
24° C	78.0	6.0	1.0	15.0
37° C	26.0	40.5	1.0	32.5
46° C	0.0	6.5	7.5	86.0

TABLE 20

AVERAGE ELONGATION OF SEEDLINGS 48 DAYS AFTER SOWING OF SEEDS

Temperature treatment for 8 hours following after-ripening	Shoot growth (cm)
Control (planted immediately)	20.0
24° C	17.6
37° C	13.7
46° C	0.0

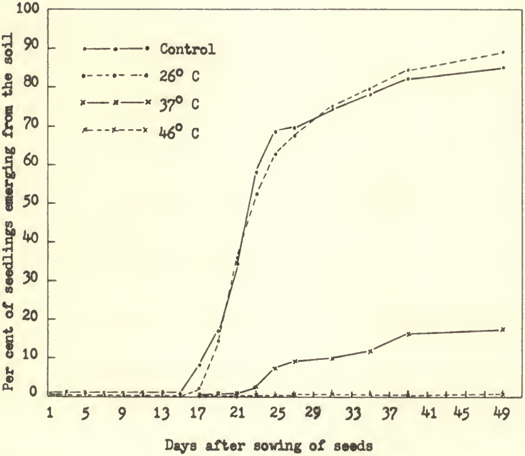

Figure 13. Effect of high temperature, following 1300 hours of after-ripening at 7° C, on seed germination

Table 21. It should also be noticed, Table 22, that the height of the seedlings decreased with increasing temperature and that with exposure to 46° C treatment rotting of the seeds increased from 86 per cent (Table 19) to 93.5 per cent.

Further investigation on the influence of increasing the chilling pretreatment from 1300 to 1600 hours on the growth capacity of the seedlings reveals (Fig. 14) that the quantitative effects of the high temperature treatment were even greater. As can be seen from the data in Fig. 14 and Tables 23 and 24, the growth capacity of the seeds and seedlings was markedly reduced by the higher temperature treatments following 1600 hours of after-ripening at 7° C. (The increase in the rotting of the seeds was taken to indicate a decrease in the growth capacity of the seeds.) Although the number of seeds germinating after the 37° C treatment is much less than in the previous tests, the results in Table 24 on the height of seedlings indicates a decrease in the growth capacity. There was also an increase in the number of seedlings emerging from the soil when compared to the 1000 and 1300 hour treatments.

Since an objection could be made as to the validity of the foregoing experiments on the basis that high temperatures following the after-ripening treatment may be killing the seeds, a further experiment was made to determine the physical conditions of the seeds following the high temperature treatments. To accomplish this, the response of the embryos to seed-coat removal was noted since it has been observed that viable peach seeds will germinate immediately

TABLE 21

THE INFLUENCE OF VARIOUS TEMPERATURES ON GERMINATION, GROWTH AND VIABILITY OF SEEDS WHICH HAD BEEN PREVIOUSLY AFTER-RIPENED FOR 1300 HOURS AT 7° C

Temperature treatment for 8 hours following after-ripening	Per cent of seeds germinated		Per cent of seeds not germinated	
	Shoots emerged from soil	Shoots not emerged from soil	Viable	Rotted
Control (planted immediately)	85.0	0.0	0.0	15.0
24° C	89.0	7.5	0.0	3.5
37° C	16.5	61.5	0.5	21.5
46° C	0.0	4.0	2.5	93.5

TABLE 22

AVERAGE ELONGATION OF SEEDLINGS 48 DAYS AFTER SOWING OF SEEDS

Temperature treatment for 8 hours following after-ripening	Shoot growth (cm)
Control (planted immediately)	15.2
26° C	14.6
37° C	9.4
46° C	0.0

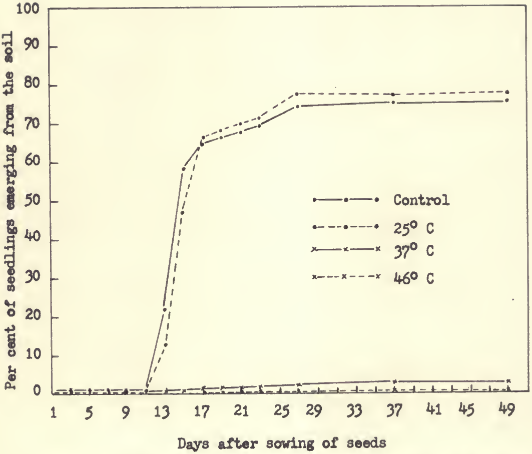

Figure 14. Effect of high temperature, following 1600 hours after-ripening at 7° C, on seed germination

TABLE 23

THE INFLUENCE OF VARIOUS TEMPERATURES ON GERMINATION, GROWTH AND VIABILITY OF SEEDS WHICH HAD BEEN PREVIOUSLY AFTER-RIPENED FOR 1600 HOURS AT 7° C

Temperature treatment for 8 hours following after-ripening	Per cent of seeds germinated		Per cent of seeds not germinated	
	Shoots emerged from soil	Shoots not emerged from soil	Viable	Rotted
Control (planted immediately)	76.0	3.0	0.0	21.0
25° C	77.5	3.5	0.0	19.0
37° C	2.0	17.5	0.0	80.5
46° C	0.0	0.0	0.0	100.0

TABLE 24

AVERAGE ELONGATION OF SEEDLINGS 48 DAYS AFTER SOWING OF SEEDS

Temperature treatment for 8 hours following after-ripening	Shoot growth (cm)
Control (planted immediately)	17.6
25° C	15.9
37° C	7.2
46° C	0.0

after this treatment (Biggs, unpublished). Coupled with this experiment, an attempt was made to determine if an additional after-ripening period, following the high temperature treatment, would again increase the growth capacity of the seedlings. The results of this experiment are recorded in Table 25. When the seeds were planted intact or without the seed coats immediately after the intermittent temperature treatment, it is obvious that these treatments affected germination. Thus, this still does not prove or disprove that the higher temperatures are killing the seeds. However, the fact that the germination of the intact seeds and the seeds without seed coats was increased by 500 hours of rechilling would indicate that the influence of sub-lethal temperatures can be reversed.

It can also be ascertained from the data in Table 25 that additional after-ripening of the seeds at 7° C, following exposure to warm temperature, increased the growth capacity of the seeds.

Since a warm temperature seems to reverse the beneficial influence of a low temperature in after-ripening peach seeds, it would seem reasonable to determine the duration of warm temperature needed for the reversal. The data on the effects of a range of temperatures of various duration on germination of seeds which had been after-ripened for 1000 hours at 7° C can be seen in Fig. 15. A comparison of the germination in the 26° C treatments indicates the degree of variability in the test since all treatments were returned to this temperature. Even with the fluctuation experienced in this test, it was obvious that an intermittent warm temperature of 30° C caused a drastic reduction in the growth capacity of the seeds. The

TABLE 25

EFFECT OF HIGH TEMPERATURE TREATMENTS, FOLLOWING 1000 HOURS OF AFTER-RIPENING AT 7° C, ON THE GERMINATION OF SEEDS

Treatment	Per cent of seeds germinating* after the designated days from sowing							
	Seeds planted immediately after the treatments				Seeds rechilled for 500 hours after the high temperature treatment before they were planted			
	16	20	24	28	4	8	12	16
Intact seeds:								
Control	10	25	35	45	0	10	100	100
26° C	0	0	25	50	0	20	80	100
37° C	0	10	15	20	0	10	30	40
46° C	0	0	0	5	0	0	0	0
Seeds with testa removed:								
Control	60	65	65	85	0	100	100	100
26° C	65	80	85	90	0	90	100	100
37° C	5	5	10	10	0	50	90	100
46° C	0	0	0	0	0	0	0	0

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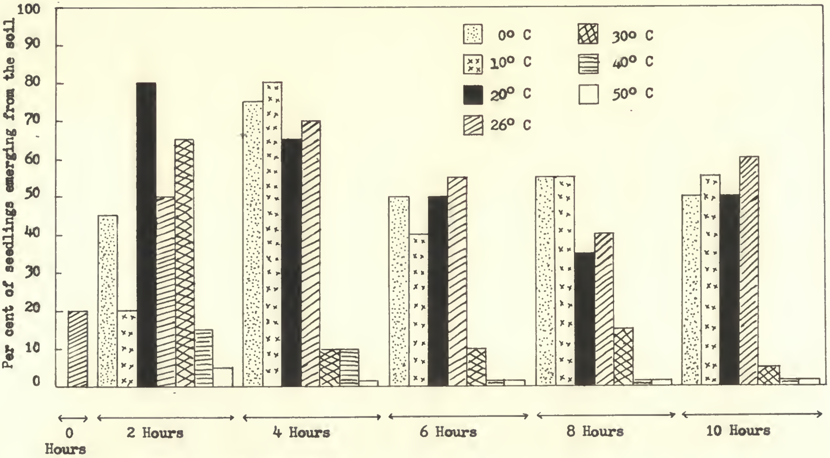
*Visible growth of the epicotyl was the criterion for germination in the case of the seeds without the seed coats.



26° C = Treatment at room temperature

Figure 15. Effect of a range of temperatures of various duration on the germination of seeds which had been after-ripened 1000 hours at 7° C

critical time of exposure to the warm temperature to cause a reduction in germination was between two and four hours.

It would seem reasonable to suggest from the foregoing experiments that a warm temperature following the after-ripening of peach seeds at a low temperature will erase the beneficial effects of the latter on the growth capacity of the seeds.

The influence of anaerobic conditions on the possible reversal of the after-ripening of seeds by a low temperature

Experiments were conducted to determine whether anaerobic conditions would reverse the effect of chilling on seed germination. It can be seen in Fig. 16 that placing seeds under a nitrogen atmosphere for eight hours slightly inhibited the number of seedlings emerging from the soil. There is a slight indication in Tables 26 and 27 that this treatment affected the growth capacity of the seedlings and not germination in a manner similar to the effects of a warm temperature. When the seeds were given an additional after-ripening at 7° C for 1000 hours following nitrogen treatment, the rate of growth was greater than the controls and the growth-inhibiting influence of nitrogen was erased.

When the exposure time to an after-ripening temperature was increased from 1000 to 1300 hours, the growth capacity of peach seeds was influenced more by anaerobic conditions. This can be seen in Fig. 17. Here again, an additional time in an after-ripening temperature erased the growth-inhibiting effects of the nitrogen.

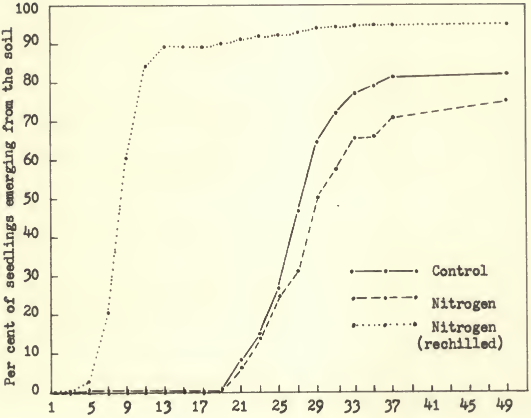


Figure 16. Effect of anaerobism on the germination of seeds after-ripened 1000 hours at 7° C

TABLE 26

THE EFFECT OF ANAEROBISM ON GERMINATION, GROWTH AND VIABILITY OF SEEDS WHICH HAD BEEN PREVIOUSLY AFTER-RIPENED FOR 1000 HOURS AT 7° C

Nitrogen treatment for 8 hours following after-ripening	Per cent of seeds germinated		Per cent of seeds not germinated	
	Shoots emerged from soil	Shoots not emerged from soil	Viable	Rotted
Control (planted immediately)	81.5	6.0	1.5	11.0
Nitrogen	75.0	7.5	1.5	15.5
Nitrogen (rechilled)	95.5	0.0	0.0	4.5

TABLE 27

AVERAGE ELONGATION OF SEEDLINGS 48 DAYS AFTER SOWING OF SEEDS

Nitrogen treatment for 8 hours following after-ripening	Elongation (cm)
Control (planted immediately)	20.0
Nitrogen	17.5
Nitrogen (rechilled)	24.3

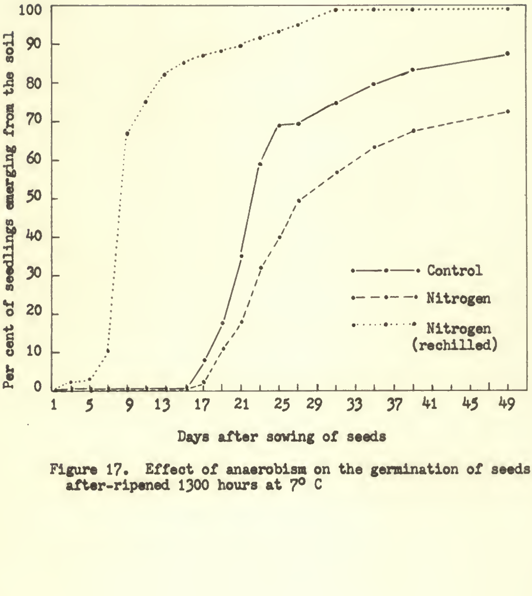


Figure 17. Effect of anaerobism on the germination of seeds after-ripened 1300 hours at 7° C

Tables 28 and 29 show further that the influence of the anaerobic condition was on the growth capacity of the seedlings and not on seed germination.

A further increase in the exposure to a chilling pretreatment from 1300 to 1600 hours caused the seeds to be even more susceptible to the nitrogen treatment as shown in Fig. 18 and Tables 30 and 31. Reduction in the growth capacity of the seeds was greater than with either the 1000 or 1300 hours of exposure to 7° C temperature prior to placing the seeds under anaerobic conditions. Although the nitrogen treatment had a more marked influence in this test, the rechilling removed the inhibition to growth.

It is suggested on the basis of these experiments that anaerobic conditions immediately following the after-ripening of seeds by low temperatures causes a reduction in the growth capacity of the seedlings but has little influence on germination.

The influence of biologically high temperatures on the possible reversal of the effects of low temperatures on the dormancy of buds

Investigations were made to determine whether or not high temperature would reverse the growth-inducing influence of a low temperature on buds. It can be seen in Fig. 19 that the various temperature treatments had only a slight influence on bud-break. The 37° C and 46° C treatment seems to have caused a more rapid rate of bud-break with the final percentage of the buds resuming growth less than the control. In this respect, the effect of 46° C is more pronounced than 37° C. Additional after-ripening at a low temperature,

TABLE 28

THE EFFECT OF ANAEROBISM ON GERMINATION, GROWTH AND VIABILITY OF SEEDS WHICH HAD BEEN PREVIOUSLY AFTER-RIPENED FOR 1300 HOURS AT 7° C

Nitrogen treatment for 8 hours following after-ripening	Per cent of seeds germinated		Per cent of seeds not germinated	
	Shoots emerged from soil	Shoots not emerged from soil	Viable	Rotted
Control (planted immediately)	85.0	0.0	0.0	15.0
Nitrogen	71.0	13.0	0.0	16.0
Nitrogen (rechilled)	99.0	0.0	0.0	1.0

TABLE 29

AVERAGE ELONGATION OF SEEDLINGS 48 DAYS AFTER SOWING OF SEEDS

Nitrogen treatment for 8 hours following after-ripening	Elongation (cm)
Control (planted immediately)	15.2
Nitrogen	14.1
Nitrogen (rechilled)	25.1

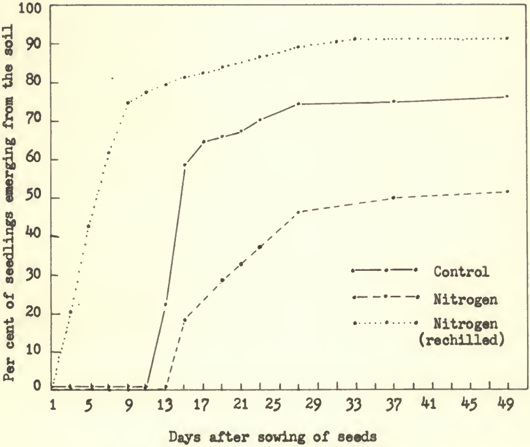


Figure 18. Effect of anaerobism on the germination of seeds after-ripened 1600 hours at 7° C

TABLE 30

THE EFFECT OF ANAEROBISM ON GERMINATION, GROWTH AND VIABILITY OF SEEDS WHICH HAD BEEN PREVIOUSLY AFTER-RIPENED FOR 1600 HOURS AT 7° C

Nitrogen treatment for 8 hours following after-ripening	Per cent of seeds germinated		Per cent of seeds not germinated	
	Shoots emerged from soil	Shoots not emerged from soil	Viable	Rotted
Control (planted immediately)	76.0	3.0	0.0	21.0
Nitrogen	51.0	9.5	0.0	39.5
Nitrogen (rechilled)	80.5	0.0	0.0	19.5

TABLE 31

AVERAGE ELONGATION OF SEEDLINGS 48 DAYS AFTER SOWING OF SEEDS

Nitrogen treatment for 8 hours following after-ripening	Elongation (cm)
Control (planted immediately)	17.4
Nitrogen	12.8
Nitrogen (rechilled)	28.3

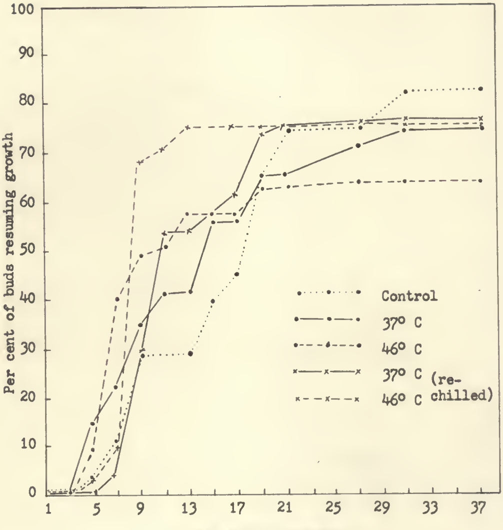


Figure 19. Effect of high temperatures on the growth of buds after-ripened for 240 hours at 7° C. (Rechilling was for 240 hours following the high temperature treatments)

following the high temperature treatments, reduced the inhibiting influence of the latter.

The influence of anaerobic conditions on the possible reversal of the effects of low temperature on the dormancy of buds

Using dormant plants that had received 240 hours of after-ripening at 7° C, the influence of anaerobic conditions on the system was studied. After the plants were subjected to a nitrogen atmosphere for eight hours, they were placed in the greenhouse where observations on the buds breaking dormancy were taken periodically. As shown in Fig. 20, anaerobic conditions caused a more rapid rate of bud-break. However, the final percentage of buds resuming growth was less than the control. An additional exposure to 240 hours of an after-ripening temperature caused a more rapid rate of bud-break and a greater percentage of the buds to break dormancy.

Discussion

It would seem from the data of this investigation that the beneficial influence of low temperatures on terminating the dormancy of peach seeds can be reversed by a relatively high temperature immediately following the chilling treatment. Furthermore, this adverse influence on seed dormancy of a relatively high temperature is not a permenant injury since subjecting the seed again to after-ripening at a low temperature will terminate dormancy. The resulting seedling seems to be a normal plant.

A rather consistant observation on the response of the seeds to a high temperature after they have been chilled was that germination

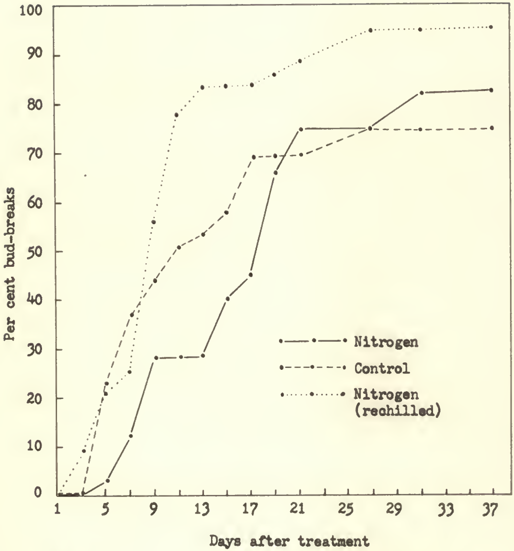


Figure 20. Effect of nitrogen treatment, following 240 hours of chilling at 7° C, on bud-breaks. (Rechilling was for 240 hours following the high temperature treatments.)

took place, but the growth capacity of the seedlings was greatly reduced. This result could be interpreted in at least two ways. Either the initial phases of germination are not affected by the high temperature treatment or the response of the system to a high temperature is a quantitative one and under the conditions of the test 100 per cent reversal was not obtained. There are indications from one test that the latter explanation seems more valid. Since there were different degrees of visible growth of the embryonic axis of seeds which had the seed coat removed and which had experienced a high temperature (Table 25), it would seem that the growth capacity of the embryos had been reduced by varying amounts.

The influence of anaerobic conditions on the reversal of the beneficial effects of low temperature on the termination of seed dormancy was strikingly similar to the influence of high temperatures. This could indicate that both of these treatments are operating through the same agent, possibly an increasing of the oxygen tension. This has been the suggested way in which a high temperature and a nitrogen atmosphere reverses vernalization (22) and similarities between the physiological responses connected with dormancy and vernalization are many.

These observations on the influence of temperature and anaerobic conditions on peach seed dormancy may have practical importance. Once peach seeds have been after-ripened, they should be kept in a cool environment and well aerated until they are planted.

If the seeds experience either a relatively high temperature or anaerobic conditions, the seeds will germinate poorly.

In contrast to the effects of a relatively high temperature and anaerobic conditions on seed dormancy, these two treatments had only a slight influence on the growth capacity of buds which had received the required duration of a low temperature to terminate dormancy. This could indicate that the systems controlling bud and seed dormancy are different.

Summary

Investigations were conducted to determine whether or not the beneficial effects of a low temperature on terminating seed and bud dormancy could be reversed by high temperatures or anaerobic conditions. The following observations were made:

1. A sub-lethal high temperature or nitrogen atmosphere can restore a dormant state to peach embryos or seedlings which had previously received the required period of chilling to terminate dormancy.

2. The dormant state re-established on embryos by a high temperature or a nitrogen atmosphere can be terminated again by after-ripening at a low temperature.

3. Four hours of high temperature, following the low temperature after-ripening of peach seeds will re-establish the dormant state to embryos.

4. A high temperature or a nitrogen atmosphere did not restore the dormancy to peach buds which had previously received the required period of chilling to terminate dormancy.

ISOLATION OF GIBBERELLIN-LIKE SUBSTANCES FROM BUDS AND SEEDS

Introduction

Since gibberellic acid (GA) has been found to break the dormancy of many plant organs and gibberellin-like substances have been isolated from many plants, an attempt was made to isolate these materials from seed and buds of peach and to determine if there was a relation between native gibberellin-like compounds and dormancy.

Materials and Methods

Extractions were made for gibberellin-like substance from dormant and non-dormant buds of Prunus persica var. Okinawa. Samples of dormant buds were comprised of apical and lateral buds plus bud scales. No attempt was made to remove the bud scales. Samples of non-dormant buds were comprised of apical meristems plus the young growing leaves, along with the first 5 to 10 mm of stem.

Extracts were also made from peach seeds var. Lovell. Several extracts were made, using 100 seeds per extract. The seeds for each extract were given different amounts of chilling, from 0 to 1200 hours, at $7^{\circ} \pm 1^{\circ}$ C.

The following isolation procedure was used with both seeds and buds. The excised material was immediately frozen at -86° C, using an acetone and dry-ice mixture. The tissues were pulverized while still frozen. Seeds were pulverized immediately after removal from the cold chamber. The pulverized tissues were extracted with redistilled methanol at 2° ± 1° C. The extraction was repeated three times at 30-minute intervals. All three filtrates obtained after treating the plant material with methanol were combined and concentrated under a partial vacuum. The extracts were stored at 0° ± 1° C in this form until just before they were to be used in the bioassay. The syrupy residue was taken up in water at pH 9.5, filtered, and the pH adjusted to 7. Following this treatment, the material was adsorbed on charcoal and eluted with 95 per cent distilled acetone. After filtering the eluate, it was concentrated to dryness under vacuum. The resulting residue was dissolved in water at pH 9.5 and filtered. This water fraction was adjusted to pH 7 and tested with the pea bioassay for gibberellin-like activity.

Before testing an extract of seeds in the Avena straight-growth bioassay, it was fractionated further by chromatographing on Whatman 3MM paper in a n-butanol-acetic acid-water (300:75:125 v/v) solvent system by the ascending technique. After the solvent had ascended to approximately 20 cm from the spot of application of the plant extract, the chromatogram was dried in an oven at 80° C for 20 minutes. The dried chromatograms were stored until they were examined by the Avena bioassay.

Bioassays

Dwarf-pea bioassay. After preliminary screening of several tests for detection of gibberellins, the following proved to be the most promising.

Pisum sativum var. Little Wonder, ten days from planting was used as the assay plant. When the seeds started germinating, approximately three days from seeding, they were transferred to a growth chamber in which light, humidity and temperature were controlled. On the tenth day from seeding, plants of uniform size were selected for the test. Twenty plants were used per treatment. The known concentrations of synthetic GA and 14 extracts made from seeds were applied to the apex of the seedlings at the rate of 50 microliters per plant. The height of the plants was measured on the seventh day from treatment.

The activity of gibberellin-like substances in extracts was estimated by comparing the elongation produced by the extracts to the elongation obtained with known concentrations of GA.

The extracts of buds were tested at two separate times under greenhouse conditions, because of the unavailability of the growth chambers at that time.

Avena straight-growth bioassay. The Avena bioassay was conducted according to the procedure outlined by Nitsch and Nitsch (68) except Avena sativa var. Vicar was used. Both growth promoters and inhibitors can be located on a chromatogram using this biological test.

Experimental Results

Preliminary experiments were first conducted to determine the size of sample and the sensitivity of the bioassay to gibberellins. Of the six extracts that were taken from peach tissue, four showed positive responses in the bioassay. A representative sample of these preliminary experiments can be seen in Table 32. In Test 1 there was activity of gibberellin-like substances in the non-dormant tissue, but in Test 2 there was no activity.

It was thought at the time that these inconsistencies were due to errors involved in establishing technique for these samples, but later experiments tended to indicate additional problems.

The activity of gibberellin-like substances in extracts made from peach buds can be seen in Table 33. In general, there appears to be no activity in most of the extracts.

The data on the response of the bioassay to seed extracts are given in Table 34. There were no positive responses from any of the extracts. On the other hand, while testing the extracts of seeds and buds it was observed that the application of extracts resulted in killing the apex of a large number of bioassay plants, or in some cases, inhibition of growth without the death of the apical tissue. This effect on the tissues was more prevalent with seed extract than with bud extract. Therefore, it is possible that growth inhibitors were present in the extracts which would have blocked the action of any gibberellin-like compounds if they were present.

In order to investigate for the presence of inhibitors which could mask the action of gibberellin-like substances, seed extracts were fractionated further by chromatography. The chromatograms were analyzed with the Avena coleoptile test. A representative sample of these data can be found in Fig. 21. It is obvious that growth inhibitors were present in the extracts. Since gibberellins would be located at R_f 0.43 to 0.62 in this system, it is possible that the inhibitors located from R_f 0.45 to 0.95 are masking growth promotors.

TABLE 32

COMPARISON OF GIBBERELLIN-LIKE ACTIVITY FOUND IN NON-DORMANT AND DORMANT BUDS

Test No. 1		Test No. 2	
Material	Average plant height (cm)	Material	Average plant height (cm)
Control (H_2O)	5.2	Control (H_2O)	7.3
Gibberellic Acid		Gibberellic Acid	
1 ppm	9.9	1 ppm	10.3
10 ppm	12.1	10 ppm	19.6
100 ppm	20.7	100 ppm	22.1
1000 ppm	24.0	1000 ppm	23.0
Non-dormant bud extract	12.3	Non-dormant bud extract	7.8
Dormant bud extract	5.1	Dormant bud extract	9.2

(Plant samples calculated 10 grams)

TABLE 33

RESPONSE OF THE DWARF-PEA BIOASSAY TO EXTRACTS FROM BUDS IN VARIOUS STAGES OF GROWTH AND DEVELOPMENT, AS COMPARED TO THAT OF PURE GIBBERELLIC ACID

Test No. 1			Test No. 2		
Dates of sampling	Treatments	Average length of seedlings	Dates of sampling	Treatments	Average length of seedlings
		(cm)			(cm)
	Control (H_2O)	7.3		Control (H_2O)	5.4
	Gibberellic acid micrograms/plant			Gibberellic acid micrograms/plant	
	0.005	8.4		0.005	6.2
	0.05	8.6		0.05	8.7
	0.5	15.0		0.5	13.3
	5.0	21.6		5.0	19.2
	50.0	24.0		50.0	17.7
1960	Extract No.:		1960	Extract No.:	
Feb. 2	1	7.2*	Apr. 12	11	6.5
9	2	7.0	19	12	6.4
16	3	7.3	26	13	5.8
23	4	7.0	May 3	14	5.8
Mar. 1	5	7.4	10	15	5.7
8	6	7.6	17	16	5.6
15	7	6.8	24	17	5.6
22	8	7.8	31	18	5.0
29	9	8.6	June 7	19	4.0
Apr. 5	10	8.6	14	20	5.2
			Sept. 26	21	5.8

*Material applied to the seedlings was equivalent to approximately 10 grams (fresh weight).

TABLE 34

THE INFLUENCE ON THE DWARF-PEA BIOASSAY OF EXTRACTS FROM SEEDS WHICH HAD RECEIVED VARYING HOURS OF PRETREATMENT AT 7° C

Treatment	Average length of seedlings (cm)
Control	5.1
Gibberellic acid micrograms/plant	
0.005	6.4
0.05	10.0
0.5	22.5
5.0	29.1
50.0	29.6
Hours at 7° ± 1° C before extraction	
0	5.3
48	3.9
96	4.6
144	4.1
192	4.3
240	4.0
288	4.3
336	4.5
384	5.0
432	4.7
624	4.7
816	4.6
1008	3.2
1200	4.9

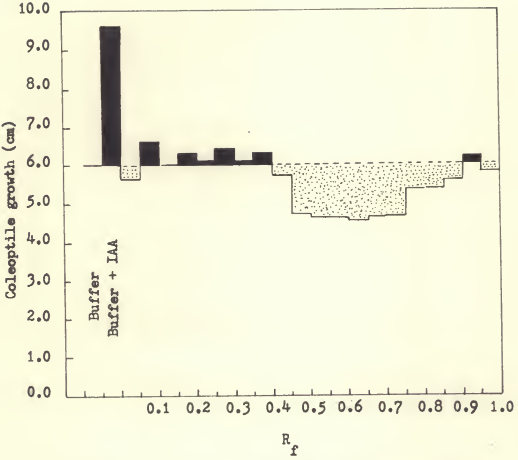


Figure 21. A histogram showing the activity of an Avena straight growth test to growth promoters and growth inhibitors on a chromatogram of seed extracts

Discussion

In the attempt to isolate gibberellin-like substances from peach seeds and buds, it was found that a great deal of variability existed in the degree of activity present in the extracts from identical materials. This made it impossible to establish whether or not there was a relation between native gibberellin-like compounds and dormancy. However, these data do indicate that gibberellin-like substances are present in peach tissue since under certain conditions gibberellin-like activity in the extracts was observed.

Recently, it has been shown that gibberellins or gibberellin-like substances occur in higher plants (25, 76). Also, the application of gibberellins to dormant organs has been found to cause a resumption of growth. From these two observations, it has been suggested that natural gibberellins may play a role in controlling dormancy. However, the results obtained in this study do not support this contention nor are they opposed to it.

There seems to be two possible explanations for the results obtained in this investigation. First, the bioassay for the gibberellin-like materials may have been inadequate; and second, there may be no relation between naturally occurring gibberellins and dormancy. At the present, a choice can not be made between these two alternatives.

To support the first suggestion, it was reported by Ikuma and Thimann (51) that they repeatedly failed to obtain gibberellin-like substances from lettuce seeds when they used the dwarf-1 maize bioassay to determine activity in the extracts. Yet, with the use of the dwarf-5 maize bioassay, they obtained gibberellin-like activity.

Thus, it is possible that the dwarf pea bioassay used in these studies did not respond to all the gibberellins present in the extract.

Another factor could have made the bioassay inadequate. It was found that growth inhibitors were present in the partially purified extracts. These materials could have counteracted any activity of gibberellin-like substances that were present in the isolated fraction. There are other reports that inhibitors were interferring with the isolation of gibberellins from plants. Corcoran and Phinney (26) reported that growth inhibitors were present in extracts from bean pods which masked the activity of gibberellins in the dwarf maize bioassay.

Summary

An attempt was made to isolate gibberellin-like substances from buds and seeds of peach and to determine if there was a relation between these substances and dormancy. It was established that gibberellin-like materials were present in peach tissue. However, a great deal of variability was observed as to the content of gibberellin-like materials in tissues from one test to another even when organs in the same physiological stage were used. This variability in the degree of activity found in the plant extracts seems to have resulted from growth inhibitors which were not separated from the gibberellin-like compounds by the fractionation procedures used. Thus, to establish whether or not the content of gibberellins can be correlated with the growth capacity of the tissue, further investigations will be needed.

SUMMARY AND CONCLUSIONS

An attempt was made to investigate the roles of plant growth regulators, especially gibberellins, in controlling the dormancy of peach. This was done by determining the influences of gibberellic acid (GA), auxins and auxin-antagonists on buds and seeds of peach in various stages of growth and development. Further, an attempt was made to isolate gibberellin-like substances from seeds and buds in different stages of growth and development to determine if there existed a correlation between such substances and dormancy. Finally, effects of high temperature and anaerobiosis on the rest period of plants were determined.

GA was effective in breaking dormancy of non-chilled as well as partially chilled buds.

In general, naphthaleneacetic acid (NAA) promoted bud-break at weaker concentrations, and inhibited at stronger concentrations. Indole-3-acetic acid (IAA) had similar effects on bud-break.

Maleic hydrazide (MH) had no particular influence on bud dormancy under the conditions of these tests.

Interactions between GA and auxins on the breaking of bud dormancy were obtained. GA and auxin used together were more effective on termination of dormancy than when used alone.

Low temperatures had a very pronounced effect on terminating bud dormancy. It was more effective in stimulating bud-growth than any of the chemicals tested. It was further observed that insufficient chilling caused buds at the base of the plants to resume growth. Intermediate doses of chilling caused a balance of terminal and lateral buds to resume growth.

Since dormancy may be hormonally controlled, the influence of exogenously applied GA, auxin and an auxin-antagonist upon the dormancy of non-after-ripened and partially after-ripened peach seeds was determined. GA induced seed germination in chilled as well as unchilled seeds.

NAA promoted seed germination at low concentrations but inhibited at high concentrations. This influence of NAA was approximately the same on seeds which had been partially after-ripened to those which had received no after-ripening at 7° C. MH had no effect on seed germination under the conditions of these tests.

No interaction between chilling or any of the chemicals tested was obtained.

Experiments were also conducted to determine whether the effect of chilling on bud and seed dormancy can be reversed by high temperatures and anaerobic conditions. It was concluded that sublethal high temperature or a nitrogen atmosphere can restore dormancy to peach embryos or seedlings which had previously received sufficient chilling to terminate dormancy.

The dormancy of embryos imposed by high temperature or nitrogen can be broken again by rechilling.

To determine whether or not native gibberellins are present in peach buds and seeds; and whether they are related to bud and seed dormancy, attempts were made to isolate gibberellin-like substances from these organs. Although such substances were found in many of the extracts, there was a great deal of variability in the occurrence or concentration of the substances present, even when identical materials and methods were used. It was found that growth inhibitors were also present in the partially purified extracts which could have counteracted the influence of the gibberellin-like substances in the bioassay. Until these inhibitors can be separated from the native gibberellin-like substances, it will be difficult to arrive at any quantitative estimates of the amount of these substances in tissues, especially by bioassay.

On the basis of the data obtained, no definite mechanism controlling dormancy was found. However, it would seem reasonable to suggest that neither gibberellins nor auxin are the material agents that are primarily responsible for peach bud or seed dormancy.

Although GA and auxins had a marked influence on the dormancy of seeds and buds, it would be reasonable to conclude that they are secondary in their influence on dormancy. Hence, it can be visualized that once the initial block to growth is removed they are then required to maintain growth and development.

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APPENDIX: DETAILED DATA TABLES

TABLE 35

THE INFLUENCE OF LOW TEMPERATURE AND GIBBERELLIC ACID ON THE TERMINATION OF BUD DORMANCY

Days of pretreatment at 7° ± 1° C	Per cent of buds broken											
	10 days after treatment				20 days after treatment				30 days after treatment			
	Control	GA			Control	GA			Control	GA		
		10^{-5}M	10^{-4}	10^{-3}		10^{-5}M	10^{-4}	10^{-3}		10^{-5}M	10^{-4}	10^{-3}
0	0.0	7.9	5.3	1.9	3.7	9.5	7.1	15.3	7.4	14.2	10.7	23.0
2	1.7	0.0	3.6	5.3	15.5	6.2	21.8	19.6	22.4	20.8	25.4	35.7
4	6.6	2.3	0.0	6.0	15.5	23.8	12.7	21.2	31.1	42.8	25.5	36.3
6	4.2	5.5	0.0	1.9	19.1	37.0	30.3	30.7	48.9	46.2	48.2	46.1
8	0.0	8.1	8.8	0.0	30.2	53.0	46.6	45.1	54.5	67.3	60.0	80.6
10	8.0	20.5	0.0	7.5	72.0	76.4	62.8	67.5	80.0	85.3	76.7	80.0
12	2.7	2.8	0.0	13.0	67.5	71.4	73.8	76.0	94.6	92.2	90.4	91.3
14	13.6	40.0	42.5	36.1	75.0	73.3	77.5	85.1	84.1	80.0	87.5	85.1
16	16.2	27.0	29.4	23.4	86.0	85.4	80.4	72.3	95.3	89.5	82.3	78.7
18	42.8	37.5	40.4	40.4	82.8	90.0	85.1	97.6	82.8	90.0	95.7	90.4
20	45.4	42.5	60.0	49.1	78.2	82.9	91.1	76.3	78.2	89.3	95.5	81.8
22	20.1	29.9	36.1	22.2	79.4	91.4	91.6	84.4	87.3	91.4	94.4	93.3

TABLE 36

THE INFLUENCE OF LOW TEMPERATURE AND NAPHTHALENEACETIC ACID ON THE TERMINATION OF BUD DORMANCY

Days of pretreatment at 7° ± 1° C	Per cent of buds broken											
	10 days after treatment				20 days after treatment				30 days after treatment			
	Control	NAA			Control	NAA			Control	NAA		
		10^{-5}M	10^{-4}	10^{-3}		10^{-5}M	10^{-4}	10^{-3}		10^{-5}M	10^{-4}	10^{-3}
0	0.0	6.1	1.9	1.6	3.7	6.1	1.9	1.6	7.4	10.2	1.9	1.6
2	1.7	0.0	6.5	13.3	15.5	8.6	6.5	22.2	22.4	22.4	23.9	26.6
4	6.6	0.0	0.0	2.0	15.5	20.7	10.9	8.3	31.1	37.7	36.3	25.0
6	4.2	0.0	0.0	2.0	19.1	4.4	16.2	18.3	48.9	37.7	51.1	36.7
8	0.0	0.0	6.3	6.6	30.2	54.7	51.0	26.6	54.5	77.3	74.4	53.3
10	8.0	3.7	22.6	5.2	72.0	67.9	60.3	68.4	80.0	83.0	77.3	76.3
12	2.7	20.0	10.8	0.0	67.5	72.5	80.4	60.9	94.6	82.5	97.8	87.8
14	13.6	33.3	27.6	25.5	75.0	78.5	76.6	76.6	84.1	83.3	89.3	80.8
16	16.2	18.3	9.8	6.6	86.0	77.5	70.5	51.1	95.3	81.6	90.2	57.7
18	42.8	24.0	51.4	27.5	82.8	84.0	82.8	75.8	82.8	96.0	82.8	89.6
20	45.4	46.3	52.3	48.1	78.2	82.9	90.4	88.8	78.2	92.6	90.4	96.3
22	20.5	31.7	39.1	28.1	79.4	78.0	78.2	87.5	87.1	85.3	91.3	93.7

TABLE 37

THE INFLUENCE OF LOW TEMPERATURE AND MALEIC HYDRAZIDE ON THE TERMINATION OF BUD DORMANCY

Days of pretreatment at 7° ± 1° C	Per cent of buds broken											
	10 days after treatment				20 days after treatment				30 days after treatment			
	Control	MH			Control	MH			Control	MH		
		10^{-5}M	10^{-4}	10^{-3}		10^{-5}M	10^{-4}	10^{-3}		10^{-5}M	10^{-4}	10^{-3}
0	0.0	5.2	0.0	0.0	3.7	5.2	0.0	0.0	7.4	7.0	6.1	4.3
2	1.7	0.0	4.3	0.0	15.5	8.5	10.8	7.6	22.4	23.4	19.5	17.3
4	6.6	9.0	0.0	2.3	15.5	15.1	15.5	16.2	31.1	27.2	31.1	39.5
6	4.2	3.8	5.2	12.5	19.1	36.5	31.5	43.7	48.9	44.2	50.0	64.5
8	0.0	21.0	6.8	8.5	30.2	76.3	59.0	61.7	54.5	84.2	79.5	63.8
10	8.0	8.5	8.1	6.5	72.0	77.1	78.3	63.0	80.0	88.5	97.3	80.4
12	2.7	13.6	11.1	3.2	67.5	75.0	77.7	60.6	94.6	79.5	88.8	73.7
14	13.6	7.2	15.3	11.6	75.0	80.0	76.9	65.1	84.1	81.8	88.4	72.0
16	16.2	22.9	16.6	22.0	86.0	79.1	71.2	72.0	95.3	79.1	78.7	82.0
18	42.8	27.2	43.4	25.9	82.8	83.6	77.3	79.6	82.8	87.2	83.0	83.3
20	45.4	44.8	46.8	54.5	78.2	75.8	74.4	88.6	78.2	86.2	89.3	93.1
22	20.1	28.0	85.7	32.7	79.4	82.0	91.4	89.0	87.1	76.0	91.4	91.4

TABLE 38

THE INFLUENCE OF GIBBERELLIC ACID AND NAPHTHALENEACETIC ACID ON THE TERMINATION OF BUD DORMANCY[1]

Concentration	Per cent of buds broken											
	Days after treatment											
	5	7	9	11	13	15	17	19	21	23	27	31
Control	4.1	12.5	29.1	29.1	29.1	41.6	45.8	66.6	75.0	75.0	83.3	83.3
NAA 10^{-4} M	9.3	15.6	15.6	34.3	37.5	56.2	59.3	71.8	71.8	71.8	75.0	75.0
NAA 10^{-3}	2.5	15.3	15.3	25.5	28.2	46.1	53.8	64.1	69.2	76.9	79.4	82.0
NAA 10^{-2}	7.4	14.8	29.6	29.6	37.0	48.1	51.8	55.5	62.9	62.9	66.6	66.6
GA 10^{-4}	5.5	8.3	11.1	11.1	11.1	16.6	30.5	52.7	66.6	69.4	77.7	77.7
" " + NAA 10^{-4}	21.4	39.2	42.8	53.5	57.1	67.8	67.8	78.5	78.5	85.7	85.7	89.2
" " + NAA 10^{-3}	5.7	28.5	41.6	48.5	51.4	68.5	77.1	82.8	82.8	82.8	88.5	91.4
" " + NAA 10^{-2}	13.8	22.4	22.4	25.0	27.7	36.1	50.0	55.5	61.1	66.6	69.4	69.4
GA 10^{-3}	11.4	17.1	25.7	25.7	34.2	54.2	57.1	68.5	71.4	71.4	80.0	80.0
" " + NAA 10^{-4}	15.6	25.0	34.3	34.3	43.7	53.1	59.3	59.3	62.5	65.5	71.8	75.0
" " + NAA 10^{-3}	3.3	23.3	33.3	33.3	36.6	56.6	56.6	70.0	73.3	73.3	76.6	76.6
" " + NAA 10^{-2}	7.7	19.2	19.2	19.2	19.2	26.9	38.4	50.0	50.0	50.0	50.0	50.0
GA 10^{-2}	28.5	42.8	53.5	64.2	67.8	78.5	78.5	78.5	78.5	78.5	78.5	78.5
" " + NAA 10^{-4}	8.3	19.4	22.2	36.1	36.1	47.2	47.2	50.0	52.7	52.7	47.2	47.2
" " + NAA 10^{-3}	18.7	34.3	43.7	43.7	43.7	50.0	53.1	53.1	56.2	56.2	56.2	56.2
" " + NAA 10^{-2}	7.4	7.4	7.4	7.4	7.4	7.4	18.5	29.6	37.0	37.0	37.0	37.0

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[1] The plants were given a chilling pretreatment of 240 hours.

TABLE 39

THE INFLUENCE OF GIBBERELLIC ACID AND MALEIC HYDRAZIDE ON THE TERMINATION OF BUD DORMANCY[1]

Concentration	Per cent of buds broken											
	Days after treatment											
	5	7	9	11	13	15	17	19	21	23	27	31
Control	4.1	12.5	29.1	29.1	29.1	41.6	45.8	66.6	75.0	75.0	83.3	83.3
MH 10^{-4} M	11.1	30.5	36.1	44.4	44.4	69.4	75.0	75.0	75.0	80.5	83.3	86.1
MH 10^{-3}	3.1	3.1	9.3	9.3	9.3	21.8	31.2	46.8	56.2	56.2	62.5	62.5
MH 10^{-2}	16.6	20.0	50.0	50.0	50.0	73.3	73.3	73.3	73.3	86.6	86.6	90.0
GA 10^{-4}	7.1	17.8	25.0	28.5	39.2	42.8	42.8	46.4	46.4	50.0	53.8	53.8
" " + MH 10^{-4}	5.0	7.5	20.0	22.5	32.5	42.5	45.0	50.0	52.5	57.5	60.0	72.5
" " + MH 10^{-3}	10.7	25.0	25.0	25.0	39.2	42.8	50.0	60.7	75.0	78.5	78.5	78.5
" " + MH 10^{-2}	12.9	25.8	38.7	38.7	38.5	45.1	54.8	54.8	61.2	61.2	64.5	80.6
GA 10^{-3}	2.7	16.6	19.4	22.2	36.1	52.7	58.3	63.8	66.6	66.6	66.6	66.6
" " + MH 10^{-4}	5.5	19.4	25.0	36.1	36.1	52.7	52.7	52.7	63.8	66.6	75.0	77.7
" " + MH 10^{-3}	11.7	32.3	35.3	41.1	44.1	55.8	55.8	61.7	67.6	67.6	73.5	76.4
" " + MH 10^{-2}	5.7	11.4	14.3	25.7	31.4	51.4	57.1	71.4	77.1	77.1	82.8	82.8
GA 10^{-2}	6.4	12.9	32.2	32.2	41.9	54.8	58.0	61.2	61.2	61.2	64.5	67.7
" " + MH 10^{-4}	9.0	18.0	36.0	39.3	48.4	60.6	72.7	72.7	75.7	75.7	78.7	78.7
" " + MH 10^{-3}	12.5	20.8	25.0	29.1	37.5	58.3	62.5	66.6	66.6	66.6	70.8	70.8
" " + MH 10^{-2}	10.7	32.1	42.8	53.5	53.5	53.5	53.5	57.1	64.3	64.3	64.3	64.3

[1] Plants were given a chilling pretreatment of 240 hours.

TABLE 40

THE INFLUENCE OF NAPHTHALENEACETIC ACID AND MALEIC HYDRAZIDE ON THE TERMINATION OF BUD DORMANCY[1]

Concentration	Per cent of buds broken											
	Days after treatment											
	5	7	9	11	13	15	17	19	21	23	27	31
Control	4.1	12.5	29.1	29.1	29.1	41.6	45.8	66.6	75.0	75.0	83.3	83.3
MH 10^{-4} M	11.1	30.5	36.1	44.4	44.4	69.4	75.0	75.0	75.0	80.5	83.3	86.1
MH 10^{-3}	3.1	3.1	9.3	9.3	9.3	21.8	31.2	46.8	56.2	56.2	62.5	62.5
MH 10^{-2}	16.6	20.0	50.0	50.0	50.0	73.3	73.3	73.3	73.3	86.6	86.6	90.0
NAA 10^{-4}	9.3	15.6	15.6	34.3	37.5	56.2	59.3	71.8	71.8	71.8	75.0	75.0
" " + MH 10^{-4}	3.1	15.6	31.2	40.6	43.7	68.7	71.8	81.2	96.8	96.8	96.8	96.8
" " + MH 10^{-3}	5.7	8.5	11.4	14.2	17.1	31.4	34.3	51.4	65.7	71.4	82.8	85.7
" " + MH 10^{-2}	0.0	0.0	10.0	10.0	20.0	35.0	45.0	55.0	60.0	70.0	85.0	85.0
NAA 10^{-3}	2.5	15.3	15.3	25.5	28.2	46.1	53.8	64.1	69.2	76.9	79.4	82.0
" " + MH 10^{-4}	7.1	14.2	14.2	14.2	28.4	35.7	57.1	64.2	85.7	85.7	85.7	85.7
" " + MH 10^{-3}	6.2	12.5	15.6	25.0	28.1	50.0	50.0	53.3	65.6	65.6	71.8	75.0
" " + MH 10^{-2}	0.0	12.5	12.5	18.7	18.7	31.2	56.2	75.0	75.0	75.0	81.2	81.2
NAA 10^{-2}	7.4	14.8	29.6	29.6	37.0	48.1	51.8	55.5	62.9	62.9	66.6	66.6
" " + MH 10^{-4}	10.0	15.0	15.0	15.0	30.0	30.0	35.0	40.0	45.0	45.0	50.0	60.0
" " + MH 10^{-3}	0.0	0.0	0.0	6.2	12.4	18.7	28.1	37.5	46.8	53.1	56.2	62.5
" " + MH 10^{-2}	8.3	12.5	25.0	33.3	37.5	41.6	50.0	58.3	62.5	62.5	62.5	62.5

[1] Plants were given a chilling pretreatment of 240 hours.

TABLE 41

THE INFLUENCE OF GIBBERELLIC ACID AND INDOLE-3-ACETIC ACID ON BREAKING THE DORMANCY OF BUDS

Concentration	Per cent of buds broken				
	Days after treatment				
	7	14	21	28	47
Control (H_2O)	0.0	3.6	25.4	36.3	50.9
GA 10^{-6} M	4.1	25.9	33.3	44.5	68.5
GA 10^{-5}	4.1	29.1	34.9	47.6	68.2
GA 10^{-4}	5.5	37.7	39.5	60.4	75.0
GA 10^{-3}	5.5	42.8	44.4	64.4	84.4
IAA 10^{-6}	3.7	10.0	29.6	33.3	68.5
IAA 10^{-5}	4.0	16.0	36.0	44.0	72.0
IAA 10^{-4}	5.4	32.4	40.5	59.4	86.4
IAA 10^{-3}	4.3	21.7	39.1	43.4	52.1
IAA 10^{-6} + GA 10^{-4}	39.5	60.4	69.7	72.0	90.6
IAA 10^{-5} + " "	28.5	62.8	65.7	68.5	74.2
IAA 10^{-4} + " "	7.8	27.4	25.4	31.3	66.6
IAA 10^{-3} + " "	1.7	25.1	26.7	26.7	26.7

TABLE 42

SEEDS GERMINATING AFTER TREATMENT WITH GIBBERELLIC ACID AND NAPHTHALENEACETIC ACID

Concentration	Per cent germination														
	Days after sowing														
	12	14	16	18	20	22	24	26	28	30	32	34	38	42	46
Control (H_2O)									0	5	5	5	10	10	10
NAA 10^{-6} M						0	5	5	10	10	15	15	20	20	20
NAA 10^{-5}							0	5	5	5	5	5	10	15	20
NAA 10^{-4}									0	5	10	10	15	20	30
NAA 10^{-3}															0
GA 10^{-6}					0	10	25	30	30	35	40	55	55	55	55
" " + NAA 10^{-6}					0	20	25	25	25	25	30	35	35	35	35
" " + NAA 10^{-5}					0	10	10	15	15	20	20	25	25	25	30
" " + NAA 10^{-4}			0	5	5	10	10	10	10	10	10	10	10	15	15
" " + NAA 10^{-3}															0
GA 10^{-5}				0	10	10	10	10	15	20	20	20	20	20	20
" " + NAA 10^{-6}					0	5	10	15	20	20	20	25	25	25	25
" " + NAA 10^{-5}			0	5	10	10	15	20	20	25	25	25	25	25	25
" " + NAA 10^{-4}				0	5	5	5	10	15	15	20	20	20	20	25
" " + NAA 10^{-3}															0
GA 10^{-4}			0	5	5	10	10	10	15	15	15	15	20	30	30
" " + NAA 10^{-6}			0	5	5	10	10	10	10	15	15	20	25	25	35
" " + NAA 10^{-5}					0	5	5	5	5	5	20	25	30	35	35
" " + NAA 10^{-4}					0	5	10	10	10	10	10	15	20	20	20
" " + NAA 10^{-3}										0	5	5	10	10	10
GA 10^{-3}			0	5	10	25	40	40	40	40	40	45	50	55	55
" " + NAA 10^{-6}		0	5	10	15	15	15	15	15	15	25	25	25	25	25
" " + NAA 10^{-5}		0	5	5	5	10	15	20	20	20	20	20	20	20	25
" " + NAA 10^{-4}					0	5	10	10	10	10	10	15	20	20	25
" " + NAA 10^{-3}										0	5	5	10	10	10
GA 10^{-2}			0	5	5	10	10	10	10	10	10	10	15	15	25
" " + NAA 10^{-6}	0	10	10	10	15	20	25	25	25	35	35	35	40	40	40
" " + NAA 10^{-5}	0	5	10	15	15	20	25	25	30	30	30	35	40	40	50
" " + NAA 10^{-4}		0	5	5	10	10	25	25	25	25	25	25	25	30	40
" " + NAA 10^{-3}												0	10	20	30

TABLE 43

SEEDS GERMINATING AFTER TREATMENT WITH GIBBERELLIC ACID AND MALEIC HYDRAZIDE

Concentration	Per cent germination														
	Days after sowing														
	12	14	16	18	20	22	24	26	28	30	32	34	38	42	46
Control (H_2O)						0	5	10	15	20	20	25	35	40	40
MH 10^{-6} M				0	5	15	25	25	25	30	30	30	30	35	35
MH 10^{-5}				0	5	5	5	5	5	10	15	20	30	30	35
MH 10^{-4}					0	5	5	5	10	10	15	15	25	30	30
MH 10^{-3}						0	5	5	10	10	10	20	20	20	20
GA 10^{-6}				0	5	5	5	5	5	5	10	10	10	15	15
" " + MH 10^{-6}				0	5	5	5	10	15	15	15	25	25	35	35
" " + MH 10^{-5}					0	5	5	10	10	10	10	15	15	15	20
" " + MH 10^{-4}					0	5	10	10	10	10	10	10	10	10	15
" " + MH 10^{-3}					0	15	20	20	30	30	30	30	30	30	30
GA 10^{-5}					0	5	5	5	10	10	10	15	15	15	15
" " + MH 10^{-6}				0	15	20	25	30	30	30	30	30	30	30	30
" " + MH 10^{-5}						0	5	5	5	5	5	10	15	15	15
" " + MH 10^{-4}			0	5	5	25	25	30	35	35	40	40	45	45	45
" " + MH 10^{-3}		0	5	5	15	15	25	25	25	25	25	25	25	30	30
GA 10^{-4}				0	10	10	15	15	15	15	15	15	20	25	25
" " + MH 10^{-6}					0	10	15	15	15	20	20	20	20	20	20
" " + MH 10^{-5}				0	5	5	5	10	15	15	15	15	15	15	25
" " + MH 10^{-4}				0	10	10	10	10	20	20	25	30	30	30	30
" " + MH 10^{-3}						0	5	5	5	10	10	15	25	25	25
GA 10^{-3}		0	5	5	5	5	5	5	5	5	15	15	15	25	25
" " + MH 10^{-6}				0	10	10	20	20	30	30	35	40	40	40	40
" " + MH 10^{-5}			0	5	10	10	15	25	25	30	40	40	40	40	55
" " + MH 10^{-4}				0	10	10	10	10	15	15	20	30	30	30	40
" " + MH 10^{-3}				0	5	5	5	5	5	5	5	10	10	10	15
GA 10^{-2}	0	5	10	15	15	25	35	40	40	40	45	45	45	55	60
" " + MH 10^{-6}			0	5	5	5	10	10	10	20	25	25	25	30	30
" " + MH 10^{-5}		0	5	5	5	10	20	25	25	30	30	35	35	40	40
" " + MH 10^{-4}	0	5	5	10	10	15	15	15	20	25	25	25	25	25	25
" " + MH 10^{-3}	0	10	10	15	15	15	20	20	20	25	30	30	30	30	30

TABLE 44

SEEDS GERMINATING AFTER TREATMENT WITH NAPHTHALENEACETIC ACID AND MALEIC HYDRAZIDE

Concentration	Per cent germination												
	Days after sowing												
	16	18	20	22	24	26	28	30	32	34	38	42	46
Control (H_2O)					0	5	15	15	20	20	20	25	25
MH 10^{-6} M										0	10	25	25
MH 10^{-5}								0	5	5	5	5	10
MH 10^{-4}			0	5	10	15	15	15	15	15	15	15	20
MH 10^{-3}								0	10	10	15	15	15
NAA 10^{-6}						0	5	5	5	10	10	15	20
" " + MH 10^{-6}		0	5	5	15	15	20	20	25	25	30	35	35
" " + MH 10^{-5}				0	5	10	10	10	20	25	25	25	25
" " + MH 10^{-4}						0	5	5	5	5	15	15	15
" " + MH 10^{-3}		0	5	5	5	5	5	10	10	15	20	25	25
NAA 10^{-5}												0	5
" " + MH 10^{-6}		0	5	10	15	15	15	15	20	20	20	20	20
" " + MH 10^{-5}		0	5	10	10	15	20	20	20	20	25	25	25
" " + MH 10^{-4}		0	5	5	5	10	10	10	10	10	15	20	20
" " + MH 10^{-3}													0
NAA 10^{-4}			0	10	15	15	15	15	15	15	20	20	20
" " + MH 10^{-6}			0	5	10	10	20	25	25	25	25	25	25
" " + MH 10^{-5}			0	5	5	5	5	10	10	20	20	20	20
" " + MH 10^{-4}		0	5	15	20	20	20	25	35	40	40	40	40
" " + MH 10^{-3}					0	10	10	15	15	15	20	20	20
NAA 10^{-3}													0
" " + MH 10^{-6}													0
" " + MH 10^{-5}										0	5	5	5
" " + MH 10^{-4}													0
" " + MH 10^{-3}								0	5	5	10	10	10
NAA 10^{-2}													0
" " + MH 10^{-6}													0
" " + MH 10^{-5}													0
" " + MH 10^{-4}													0
" " + MH 10^{-3}													0

TABLE 45

EFFECT OF GIBBERELLIC ACID AND NAPHTHALENEACETIC ACID ON SEEDS GERMINATING AFTER AFTER-RIPENING AT 7° C FOR 1000 HOURS

Concentration	Per cent germination										
	Days from sowing										
	11	13	15	17	19	21	23	25	27	29	31
Control (H_2O)											0
NAA 10^{-6} M					0	5	10	15	20	30	30
NAA 10^{-5}					0	5	10	20	30	30	30
NAA 10^{-4}					0	5	5	15	15	20	20
NAA 10^{-3}											0
GA 10^{-6}							0	5	5	5	5
" " + NAA 10^{-6}							0	20	20	20	25
" " + NAA 10^{-5}							0	5	5	10	10
" " + NAA 10^{-4}							0	10	10	10	10
" " + NAA 10^{-3}											0
GA 10^{-5}					0	5	5	10	20	20	20
" " + NAA 10^{-6}					0	5	10	5	5	10	10
" " + NAA 10^{-5}					0	5	10	10	10	10	10
" " + NAA 10^{-4}							0	15	20	25	25
" " + NAA 10^{-3}											0
GA 10^{-4}								0	15	15	25
" " + NAA 10^{-6}						0	5	10	20	20	20
" " + NAA 10^{-5}					0	5	5	10	25	30	35
" " + NAA 10^{-4}						0	10	15	15	15	25
" " + NAA 10^{-3}											0
GA 10^{-3}					0	5	5	5	15	15	15
" " + NAA 10^{-6}							0	15	30	30	30
" " + NAA 10^{-5}						0	10	15	20	25	25
" " + NAA 10^{-4}							0	10	10	15	20
" " + NAA 10^{-3}									0	5	5
GA 10^{-2}					0	10	10	15	20	20	25
" " + NAA 10^{-6}					0	10	15	30	55	55	65
" " + NAA 10^{-5}						0	5	15	20	25	30
" " + NAA 10^{-4}					0	5	5	10	10	10	15
" " + NAA 10^{-3}								0	10	10	15

TABLE 46

EFFECT OF GIBBERELLIC ACID AND MALEIC HYDRAZIDE ON SEEDS GERMINATING AFTER AFTER-RIPENING AT 7° C FOR 1000 HOURS

Concentration	Per cent germination											
	Days after sowing											
	9	11	13	15	17	19	21	23	25	27	29	31
Control (H_2O)							0	10	15	30	35	35
MH 10^{-6} M					0	10	30	60	65	70	70	75
MH 10^{-5}					0	15	45	60	65	65	65	70
MH 10^{-4}				0	5	15	20	45	50	60	60	75
MH 10^{-3}						0	15	20	35	40	45	50
GA 10^{-6}					0	5	5	20	40	40	50	55
" " + MH 10^{-6}				0	5	5	40	55	65	70	75	75
" " + MH 10^{-5}					0	10	25	35	65	75	80	85
" " + MH 10^{-4}					0	5	15	30	45	55	70	70
" " + MH 10^{-3}						0	15	30	45	50	50	55
GA 10^{-5}				0	5	15	25	40	70	65	85	85
" " + MH 10^{-6}					0	10	15	35	55	70	80	80
" " + MH 10^{-5}					0	10	10	35	55	55	60	75
" " + MH 10^{-4}				0	5	5	20	50	55	55	60	70
" " + MH 10^{-3}					0	10	20	40	45	55	65	65
GA 10^{-4}			0	5	5	20	30	40	60	80	85	85
" " + MH 10^{-6}						0	5	40	45	45	50	55
" " + MH 10^{-5}					0	15	25	30	45	50	65	70
" " + MH 10^{-4}				0	5	10	15	30	35	50	65	70
" " + MH 10^{-3}					0	10	25	35	55	55	55	60
GA 10^{-3}			0	5	5	30	40	55	60	60	75	75
" " + MH 10^{-6}			0	10	10	20	25	45	60	65	70	70
" " + MH 10^{-5}			0	5	5	20	30	45	50	50	60	65
" " + MH 10^{-4}					0	10	15	40	60	70	75	75
" " + MH 10^{-3}					0	35	35	45	60	60	60	60
GA 10^{-2}		0	5	5	10	40	40	45	55	75	80	85
" " + MH 10^{-6}	0	5	5	5	15	30	40	60	60	65	70	80
" " + MH 10^{-5}			0	5	15	25	40	60	65	65	70	70
" " + MH 10^{-4}		0	5	10	15	20	30	45	50	55	55	65
" " + MH 10^{-3}	0	5	10	10	30	30	45	65	70	70	75	75

TABLE 47

EFFECT OF NAPHTHALENEACETIC ACID AND MALEIC HYDRAZIDE ON SEEDS GERMINATING AFTER AFTER-RIPENING AT 7° C FOR 1000 HOURS

Concentration	Per cent germination										
	Days after sowing										
	11	13	15	17	19	21	23	25	27	29	31
Control (H_2O)							0	10	25	35	40
MH 10^{-6} M						0	35	55	65	65	65
MH 10^{-5}						0	25	35	50	60	60
MH 10^{-4}				0	10	25	40	65	65	70	70
MH 10^{-3}				0	10	10	40	55	60	60	70
NAA 10^{-6}				0	10	20	25	30	45	60	60
" " + MH 10^{-6}			0	5	5	20	30	55	60	65	70
" " + MH 10^{-5}					0	20	25	45	45	60	60
" " + MH 10^{-4}			0	5	5	20	30	55	60	65	65
" " + MH 10^{-3}				0	10	10	25	35	50	55	55
NAA 10^{-5}			0	5	10	25	35	40	55	65	65
" " + MH 10^{-6}				0	10	40	60	65	70	75	85
" " + MH 10^{-5}					0	10	35	50	50	55	55
" " + MH 10^{-4}					0	15	35	55	55	65	65
" " + MH 10^{-3}		0	5	5	30	50	65	70	70	75	75
NAA 10^{-4}				0	5	15	40	55	65	65	65
" " + MH 10^{-6}			0	5	25	45	50	60	75	75	75
" " + MH 10^{-5}		0	5	5	10	30	45	65	70	70	75
" " + MH 10^{-4}				0	10	15	40	40	50	60	60
" " + MH 10^{-3}				0	5	35	50	55	60	70	70
NAA 10^{-3}					0	5	5	15	30	30	30
" " + MH 10^{-6}						0	5	5	10	20	20
" " + MH 10^{-5}						0	5	5	5	5	5
" " + MH 10^{-4}				0	5	5	5	5	15	15	15
" " + MH 10^{-3}							0	5	20	30	30
NAA 10^{-2}											0
" " + MH 10^{-6}											0
" " + MH 10^{-5}											0
" " + MH 10^{-4}											0
" " + MH 10^{-3}											0

BIOGRAPHICAL SKETCH

The author, Karan Singh Chauhan, was born on February 12, 1934, in the district of Saharanpur, India. He received his elementary education in a village school and passed the matriculation examination of Panjab University in March, 1951. He studied for two years at B. R. College, Agra, and in 1953 transferred to Govt. Agri. College, Kanpur (affiliated with Agra University), from which he received the degrees of Bachelor of Science in Agriculture, with horticulture as one of the major subjects, in 1955, and Master of Science in Horticulture in 1957.

While studying at Agra and Kanpur, he was a member of National Cadet Corps, and attained the rank of Senior Under Officer (Cadet Adjutant). He also won the "All India Burdwan Shooting Trophy" in 1956.

In June, 1957, he was married to Savitri Devi and they have one child, a daughter.

He entered the University of Florida, Gainesville, in February, 1958. During the period of his studies, he was a research assistant in the Department of Fruit Crops. He completed his work toward the degree of Doctor of Philosophy in the Department of Fruit Crops in August, 1961.

This dissertation was prepared under the direction of the Chairman of the Candidate's Supervisory Committee and has been approved by all members of the committee. It was submitted to the Dean of the College of Agriculture and to the Graduate Council and was prepared as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

August, 1961

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